



Journal of Fish Biology (2010) **76**, 280–300

doi:10.1111/j.1095-8649.2009.02482.x, available online at www.interscience.wiley.com

Comparative energetics and physiology of parental care in smallmouth bass *Micropterus dolomieu* across a latitudinal gradient

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The energetic and physiological status of parental smallmouth bass *Micropterus dolomieu* was investigated across the majority of their latitudinal range at the onset and near the end of care. Variables such as tissue lipid stores, plasma indicators of nutritional status and chronic stress and white muscle were used to define energetic and physiological status. Results showed that northern males (48° N) were larger and heavier than mid-northern (44° N) and southern (36° N) latitude males. For a given body size, northern males had greater whole-body lipid across the parental care period and tended to feed more (based on gut contents) than mid-northern and southern latitude conspecifics. Indicators of nutritional status were also highest in northern males. Conversely, the southern males exhibited the greatest capacity for biosynthesis across the entire parental care period as indicated by the highest level of nucleoside diphosphate kinase activities. Collectively, these findings suggest that the energetic costs and physiological consequences of care vary across latitudes, providing some of the first mechanistic evidence of how environmental conditions can influence both the ecological and physiological costs of reproduction for wild animals during parental care. The data also suggest that lake-specific processes that can vary independently of latitude may be important, necessitating additional research on fish reproductive physiology across landscapes.

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Key words: biosynthetic capacity; condition; energy indices; nucleoside diphosphate kinase; nutritional physiology; stomach content.

INTRODUCTION

The cost of reproduction is considered one of the most important life-history trade-offs (Williams, 1966; Reznick, 1992), particularly when a limited amount of resources must be allocated to growth, maintenance and reproduction. This is often termed the physiological cost of reproduction (Roff, 2002). Alternatively, reproductive costs

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can also be ecological and involve interactions with the external environment, where reproductive activities make individuals more prone to threats such as disease or predation (Reznick, 1992). Much work has explored how individuals balance the physiological costs of reproduction by adopting different reproductive strategies (Gross & Charnov, 1980), but little work has focused on how reproductive investment and the costs of reproduction change in different environments. In addition, most animal groups exhibit some form of specialized parental care behaviours (e.g. mammals, birds and a proportion of fishes, reptiles and invertebrates; Clutton-Brock, 1991). The parents already face a suite of potential consequences such as loss of mass (Townsend, 1986; Moreno, 1989; Marconato *et al.*, 1993), depletion of energy stores (Fitzgerald *et al.*, 1989; Gillooly & Baylis, 1999; Mackereth *et al.*, 1999) and reduced future breeding opportunities (Coleman *et al.*, 1985) associated with the provision of care. As such, environmental variation has the potential to further influence reproductive investment and costs during this already challenging time. Changes in the costs of parental care associated with different environments have been confirmed by only a handful of taxon-specific empirical studies (Boggs & Ross, 1993; Delope *et al.*, 1993; Jones & Reynolds, 1999; Green & McCormick, 2005; Hale & St Mary, 2007). These studies have considered how one particular environmental variable influences the investment or costs of parental care, such as adult food limitation (Boggs & Ross, 1993), parasite load (Delope *et al.*, 1993) or in the aquatic environment, low oxygen conditions (Jones & Reynolds, 1999; Green & McCormick, 2005) and salinity (Hale & St Mary, 2007). No work has yet examined how combined environmental effects, such as those produced by latitudinal differences in climate, could influence reproductive investment or the cost of reproduction.

Latitude and associated differences in seasonality have broad-scale environmental implications. Patterns of latitude and energy availability are well established (Currie, 1991) and have been used most often to explain patterns in species richness and abundance (Currie & Fritz, 1993; Davies *et al.*, 2007; Buckley *et al.*, 2008). Differences in seasonality and available energy also have the ability to influence how certain animals balance growth and energy acquisition [e.g. birds (Blem, 1976); fishes (Schultz & Conover, 1997; Yamahira & Conover, 2002)]. In particular, most fish groups show seasonal patterns in energy stores (Love, 1970; Adams *et al.*, 1982; Reznick & Braun, 1987), and individuals in harsh environments find their energy stores more depleted after winter than conspecifics in milder environments (Meffe & Snelson, 1993; Schultz & Conover, 1997; Billerbeck *et al.*, 2000). In some systems, individuals will often make a trade-off between growth and reproduction along a latitudinal gradient, where northern populations invest more in growth and delay reproduction, whereas southern populations will reproduce at a younger age and smaller size (Heibo *et al.*, 2005; Blanck & Lamouroux, 2007). As energy reserves are essential to many fundamental activities, including reproduction and survival, these latitudinal changes in seasonality may have important implications for an animal that has seasonal patterns in energy stores, inhabits a wide range of latitudes and provides lengthy and energetically costly parental care such as the teleost smallmouth bass *Micropterus dolomieu* Lacépède. As *M. dolomieu* populations extend northward, modelling studies (Shuter *et al.*, 1980; Garvey & Marschall, 2003) predict that fish need to (1) invest more energy into lipids and gonads, typically by autumn of the previous year and (2) be more selective about investing energy into

reproduction. Indeed, the ultimate northern limit to the range of any organism should be driven by energetic limitations (Currie, 1991).

The native range of *M. dolomieu* encompasses much of eastern and central North America (Scott & Crossman, 1973). Over the past century, *M. dolomieu* have been widely introduced into other bodies of water as a game fish and now range from the southern U.S.A. to north-western Ontario and Québec (MacCrimmon & Robbins, 1975) thus extending across 15° of latitude. Within this distribution, *M. dolomieu* experience variable environmental conditions where some regions have very short mild winters while others have long and severe winters. Reduced feeding opportunities (due to a period of quiescence) during the winter (Lemons & Crawshaw, 1985; Kolok, 1991) can thus add an additional energetic constraint on parental fish. In the spring when water temperatures reach *c.* 15° C, male *M. dolomieu* move into the littoral zone, create saucer-shaped depressions in the substratum with their caudal fin, court and spawn with females, and then provide sole care for up to 6 weeks (Scott & Crossman, 1973; Ridgway, 1988). Providing care for this extended period of time has been shown to have important physiological and energetic implications for the nesting male, including increased cardiac output (Cooke, 2004), loss of energy stores (Gillooly & Baylis, 1999; Mackereth *et al.*, 1999) and changes in nutritional condition (Hanson & Cooke, 2009). Furthermore, there is some evidence that guarding males do not actively feed during the parental care period and must rely on endogenous energy stores (Hinch & Collins, 1991; Mackereth *et al.*, 1999) for the energetically costly activities such as egg fanning and chasing nest predators.

The goal of this study was to examine how broad-scale environmental patterns associated with latitude influence reproductive investment and associated costs of parental care using *M. dolomieu* as a model. To do so, *M. dolomieu* were sampled from lakes at three latitudes. The first hypothesis tested for evidence of latitudinal variation in initial energy status of *M. dolomieu* providing care. Here, two predictions are possible; modelling studies (Shuter *et al.*, 1980; Garvey & Marschall, 2003) predict that fish in northern clines should invest more energy into reproduction such that energy levels are higher at the initiation of care. Conversely, fish from higher latitudes are exposed to longer and harsher winters which could lead to them possessing lower initial energy stores, being in poorer nutritional condition and exhibiting signs of stress. The second hypothesis tested whether the magnitude of change in energetic and physiological status of parental males across the parental care period was influenced by latitude. Magnitude of change should be independent of latitude because males must perform the same energetically costly activities at similar temperatures (*i.e.* spawning begins when water temperature is near 15°C at all latitudes; Ridgway, 1988) and with limited opportunities for feeding (Hinch & Collins, 1991). The third hypothesis tested for the presence of differential trade-offs between individual growth and reproduction across latitudes. Northern fish are expected to show evidence for greater trade-offs between growth and reproduction and thus demonstrate lower potential for growth during the parental care period. An indicator of biosynthetic capacity, nucleoside diphosphokinase (NDPK), was used to test for differences in the maintenance of growth potential across the period of parental care.

MATERIALS AND METHODS

STUDY SITES AND SUBJECTS

Male *M. dolomieu* were collected from five lakes across North America [Bull Shoals Lake, MO–AR, U.S.A. ($36^{\circ} 25' N$; $92^{\circ} 45' W$) and Table Rock Lake, MO, U.S.A. ($36^{\circ} 35' N$; $93^{\circ} 50' W$) 20 April to 14 May 2007, Charleston Lake ($44^{\circ} 32' N$; $76^{\circ} 00' W$) and Big Rideau Lake, Ontario, Canada ($44^{\circ} 46' N$; $76^{\circ} 13' W$) 25 May to 9 June 2007, and Rainy Lake, Ontario, Canada ($48^{\circ} 38' N$; $93^{\circ} 15' W$), 8 to 21 June 2007] (Fig. 1). These locations were selected in order to represent the extremes of the latitudinal range of *M. dolomieu* (Scott & Crossman, 1973; Mandrak & Crossman, 1992). Average winter temperature (from December to March) at $36^{\circ} 21' N$ is $4.1^{\circ} C$, $-6.1^{\circ} C$ at $44^{\circ} 44' N$ and $-10.8^{\circ} C$ at $48^{\circ} 43' N$ [National Oceanic and Atmospheric Administration (NOAA); <http://www.ncdc.noaa.gov/normals.html>]. Lakes at the most southern latitude do not experience ice-cover and these areas receive only trace amounts of snow (NOAA). Associated with latitude are many other factors that could influence the ecology and chemistry of the different lakes including geology, productivity, fish community and food web structure. It is not possible to address all of these factors in this study and they are simply considered to be part of the suite of changes associated with latitude. Experimental mesocosm studies would be needed to tease out the relative influence of these and other factors.

Sampling occurred within a single spawning season (by progressively moving northward) to eliminate inter-year variability. Fish were sampled at two lakes (with the exception of the northern site) in order to provide some replication at a given latitude. For the purpose of analyses these lakes were considered separately as there were insufficient lake replicates at each latitude to include latitude as a main effect in the statistical models. Scientific collection permits were obtained from the relevant natural resource agency (Missouri Department of Conservation and Ontario Ministry of Natural Resources) and animal care protocols were approved by the Canadian Council on Animal Care. Upon arrival at each study site, snorkelling surveys were completed in order to identify *M. dolomieu* nests ($n = >30$) with fresh eggs.



FIG. 1. Geographic location of lakes sampled for parental *Micropterus dolomieu*, Bull Shoals Lake and Table Rock Lake (●), Charleston Lake and Big Rideau Lake (●) and Rainy Lake (○). The distance between the southern site latitude and mid-northern latitude site (8° latitude, c. 900 km north-south) is greater than that from the mid-northern latitude site to the most northern site (4° latitude, c. 450 km north-south).

Water temperatures were between 14.8 and 15.5° C at all locations when nests were identified and when fish were sampled on fresh eggs. Nests were marked with labelled polyvinyl chloride (PVC) tiles that were removed at the end of parental care. Among these nests, 10 were randomly selected and the males were angled using heavy fishing equipment and landed in <30 s. Previous studies have revealed that if *M. dolomieu* are landed and blood sampled in *c.* <2 min, the physiological changes in the fish associated with exercise and handling are not yet reflected in the blood and reflect the condition of the fish prior to their capture (*e.g.* cortisol levels are typically barely detectable and at lower levels than can be obtained by holding fish in sensory deprivation chambers; Hanson *et al.*, 2009). Fish were immediately transferred to a foam-lined trough filled with fresh lake water for hook removal and blood sampling. Fish were placed in a supine position for blood sampling *via* caudal venipuncture using a vacutainer (10 ml, lithium–heparin anticoagulant, Becton-Dickson; 21 G, 1½ syringe; www.bd.com). The filled vacutainer was immediately placed in a water–ice slurry and stored for up to 2 h. Blood was later centrifuged at 10 000 *g* for 7 min (Clay Adams Compact II Centrifuge, Corning; www.analyticalsci.com). Plasma vials were then placed in liquid nitrogen until transferred to a –80° C freezer until analysis. Fish were euthanized by cerebral percussion immediately after blood sampling. Axial and pectoral muscle samples were taken using a disposable sterile scalpel, wrapped in foil and placed immediately in liquid nitrogen until they could be transferred to a –80° C freezer. Sampling time (from hook removal to muscle being placed in liquid nitrogen) took between 3 and 8 min. Fish were then measured for total length (L_T , mm) and the carcass was sealed in a plastic bag (to prevent dehydration) on ice for up to 8 h. They were then transferred to a chest freezer until dissection (up to 4 days).

The remaining marked nest sites ($n = c.$ 20) were visited twice a week by snorkellers to monitor fry development. When fry are pigmented, free swimming around the nest area and exogenously feeding (*c.* 2–4 weeks after egg deposition) (Ridgway, 1988), the parental care period is nearly complete. This parental care stage is referred to as free-swimming fry (fsf). It is at this time that another 10 males were collected from each lake following the same procedures outlined above. Water temperature was between 16 and 18° C during this sampling time.

DISSECTIONS

Fish were thawed, weighed (total mass, M_T , g) and eviscerated. Stomach contents were identified when possible and weighed. Fulton's condition factor (K) was calculated from: $K = 100 M_T L_T^{-3}$ (Nash *et al.*, 2006). Liver and gonads were weighed and expressed as a percentage of M_T in order to measure the hepato-somatic index (I_H) and the gonado-somatic index (I_G). Liver lipid is one of the main forms of energy storage for many fish species, including *M. dolomieu* and reduction in the relative mass liver can indicate loss of stores (Love, 1970; Heidinger & Crawford, 1977). Similarly, individuals investing in reproduction will increase mass of gonads relative to the rest of the body (Love, 1970) and the maintenance of I_G is believed to be costly and difficult to sustain during parental care (Mackereth *et al.* 1999). The eviscerated carcass was then homogenized in a manual stainless steel meat grinder for lipid extractions.

ENERGY STORES

Eviscerated carcass (F_E) and liver (F_L) lipid content were measured using a methanol–chloroform extraction following a modified Bligh and Dyer technique (Bligh & Dyer, 1959; Smedes & Askland, 1999). Briefly, homogenized tissues (2 g) were placed in a drying oven at 80° C overnight (16–20 h) until a constant mass was attained (± 0.0005 g). The dried sample was crushed into a fine powder using a glass pestle. Two replicates of dried samples 0.20 ± 0.02 g (mean \pm s.e.) were measured for lipids. Samples were first dissolved in a 1:2:0.8 ratio of chloroform, methanol and water and then extracted with a 2:2:1.8 ratio of chloroform, methanol and water, where lipid extracts were evaporated and weighed. A second extraction was performed with a 10% methanol in chloroform solution. Total lipid content (F) for wet mass was then calculated by converting dry mass lipid values to wet mass lipid values $M_I = 100M_D(100-W_I)^{-1}$ and $L_W = 100L_D M_I^{-1}$, where M_I is the initial wet mass of sample,

M_D is the dry mass of the sample, W_I is water content, L_W is the per cent lipid of the wet sample and L_D is the per cent lipid of the dry sample. Finally, all per cent lipid values were converted into g kg^{-1} of lipid. A mean value was taken from the duplicates and the difference between replicates was never $>3\%$.

Due to a limited amount of tissue from most livers, the remaining whole-body constituents (water, protein and trace minerals) were only measured from the eviscerated carcass. Methods followed those outlined by Crossin & Hinch (2005). Another sub-sample of the homogenized tissue (2 g) was dried at 80°C overnight (16–20 h) until a constant mass was attained (± 0.0005 g). Samples were weighed and then placed in a muffle furnace ($500\text{--}600^\circ\text{C}$) for 2 h for combustion. The remaining ash was weighed in order to determine the per cent trace minerals by wet mass. The per cent body protein (C_P) was then calculated from $C_P = 100 - (C_W + C_A + C_L)$, where C_W , C_A and C_L , represent the per cent water, ash and lipid, respectively (Crossin & Hinch, 2005). Total energy density (D_T) was calculated by summing the total energy density available from lipid and the total energy density available from protein: $D_T = fD_L + pD_P$, where f is the fraction of lipid, D_L is the energy density of lipid (g kg^{-1}), p is the fraction of protein and D_P is the energy density of protein (Breck, 2008). Lipid and protein energy densities used were those established for fishes by Brett & Groves (1979); 0.0362 MJ g^{-1} for lipid and 0.0201 MJ g^{-1} for protein.

PLASMA PHYSIOLOGICAL INDICATORS

These analyses were conducted on a focal lake at each latitude; Bull Shoals Reservoir in the south, Big Rideau Lake at mid-northern and Rainy Lake in the north. Samples were analysed for concentrations of known nutritional indicators [total protein (P), cholesterol (C) and triglycerides (T)] and chronic stress [chloride (Cl), sodium (Na) and glucose (G)] (Wagner & Congleton, 2004; Hanson & Cooke, 2009) on parental *M. dolomieu* ($n = 10$ at the egg stage and $n = 10$ at the free-swimming fry stage). All assays were performed on a Roche Hitachi 917 analyser (www.roche.com).

ENZYME ACTIVITY AND PROTEIN CONCENTRATION

These analyses were conducted on a focal lake at each latitude (*i.e.* Bull Shoals Reservoir in the south, Big Rideau Lake at mid-northern and Rainy Lake in the north). Axial muscle nucleoside diphosphate kinase (NDPK) was measured as an indicator of biosynthetic capacity and growth ($n = 10$ at the egg stage and $n = 10$ at the free-swimming fry stage). This enzyme has been reported to have a positive relationship with growth in length, growth in mass and condition factor (Couture *et al.*, 1998). Axial tissue samples were thawed on ice, weighed and diluted 10-fold with homogenizing buffer. Tissue samples were homogenized in a Ultra Turrax T25 homogenizer (Janke & Kunkel; www.ika.net) with $14\text{ cm} \times 0.75\text{ cm}$ probe at maximal speed (50 g) in test tubes constantly immersed in ice as in Couture *et al.* (1998). Substrate and cofactor concentrations were determined from assay optimization. NDPK (E.C. 2.7.4.6) activities were measured as in Couture *et al.* (1998) with the following modifications, 2.0 mM ATP and 2.2 mM phosphoenolpyruvate. NDPK activities, with replicates, were measured during 5 min trials by a temperature-controlled ultraviolet and visible spectrophotometer (Varian Cary 100; Varian Inc.; www.varianinc.com) at 20°C with assay volume of 1 ml. Muscle protein concentrations (P_M) were determined as in Audet & Couture (2003) and were determined against a bovine serum albumin standard curve.

STATISTICAL ANALYSIS

All analyses were performed in JMP 7.0.1 (SAS Institute Inc.; www.sas.com) and the level of significance (α) for all tests was 0.05. All values are means \pm s.e. unless otherwise indicated. Data were tested for normality and heterogeneity of variance prior to analyses and non-normal data were \log_{10} or $\log_{10}(x + 1)$ transformed. The non-parametric Kruskal–Wallis test was used to compare K across latitudes and non-parametric multiple comparisons were performed (Zar, 1999). Briefly, this involved calculating a new standard error, which was

then used to determine the Q statistic between ranked means [$Q = (R_B - R_A)x^{-1}$; where R_B is ranked mean B , R_A is ranked mean A and x is s.e. (Zar, 1999)]. In order to determine the differences in fish body size across lakes, a one-way ANOVA followed by Tukey's honestly significant difference (HSD) *post hoc* multiple comparison was used. In order to determine the differences in physiological variables between lakes, between the parental-care stages and across the parental-care period within lakes, two-way ANOVA were performed. When tests were statistically significant, they were followed by planned comparisons in order to separate the overall effects of lake and offspring development period. For instances where there existed a strong relationship between body size and a physiological variable, the residuals from the regression between L_T and the physiological variable were used as the response variable for the two-way ANOVA. All figures are presented with lakes placed in order of increasing latitude.

RESULTS

MALE *M. DOLMIEU* L_T I_g AND I_H

L_T and K differed between lakes (L_T : ANOVA, d.f. = 4, 95, $P < 0.001$; K , Kruskal–Wallis, d.f. = 4, $P < 0.001$). The samples of parental males from northern and mid-northern latitude lakes had similar L_T and were larger than southern fish [Fig. 2(a)]. Similarly, northern males showed higher K than southern-latitude and mid-northern latitude fish were intermediate [Fig. 2(b)]. I_G differed across lakes, the offspring development period and the offspring development period within lakes [Table I and Fig. 3(a)]. Northern fish had the highest I_G and maintained I_G levels across the care period. Southern and mid-northern latitude fish showed similar I_G to northern males when guarding offspring at the egg stage but had significant decreases when brood development reached the free-swimming fry stage. I_H differed across lakes and the offspring development period [Table I and Fig. 3(b)].

ENERGY STORES, STOMACH CONTENTS AND PLASMA INDICATORS

F_E of parental *M. dolomieu* differed between lakes [Table I and Fig. 4(a)]. Northern parental *M. dolomieu* from Rainy Lake had greater lipid reserves than southern and mid-northern latitude fish for a given body size across the entire period of parental care. D showed a similar pattern [Table I and Fig. 4(b)]; northern fish showed increased positive residuals between energy density (in MJ) and L_T . F_L only differed between lakes (Table I) and northern fish showed the highest F_L values [Fig. 4(c)]. Parental male stomach content wet mass (M_S) did not differ across the offspring development stage (Table I) but did differ between lakes [Table I and Fig. 4(d)], with northern fish demonstrating the greatest M_S .

Indicators of nutritional status differed between lakes but not across the offspring development period, nor across the offspring development period within lakes (Table I). Parental males from Rainy Lake showed the highest protein and cholesterol concentrations (Fig. 5). Only one indicator of chronic stress (Na) differed between lakes, the offspring developmental stage and across the developmental stage within lakes, whereas Cl and glucose did not differ significantly in any groups (Table I). Na was the lowest for males guarding eggs for all latitudes and remained at low concentrations for northern and mid-northern latitude fish guarding the free-swimming fry stage, while southern fish showed a significant increase (Fig. 6).

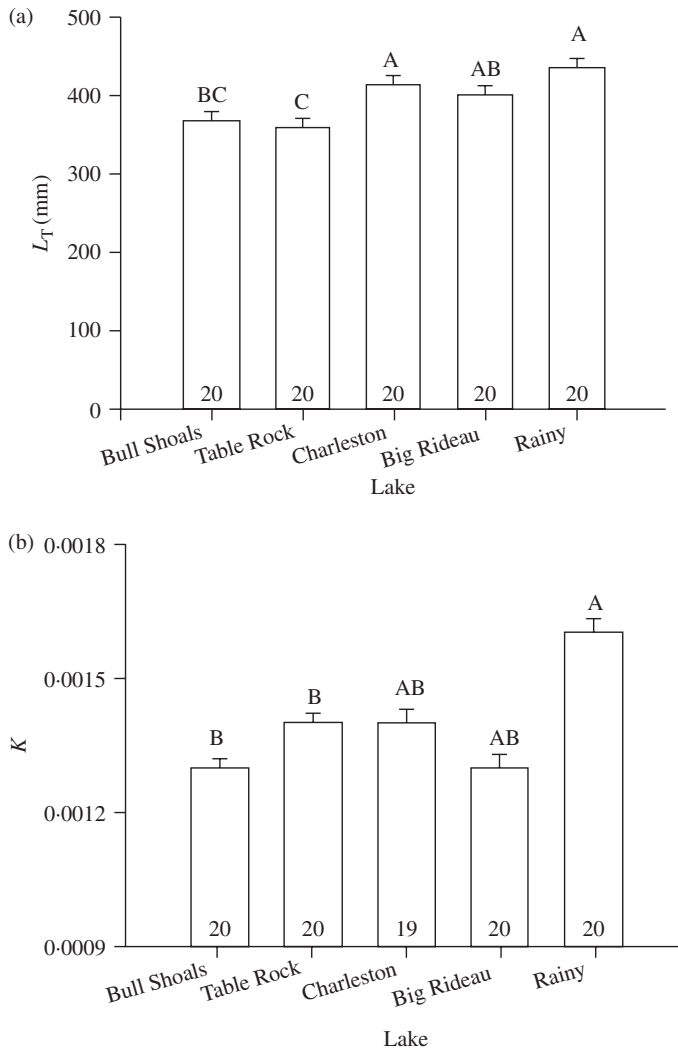


FIG. 2. Mean \pm s.e. (a) total length (L_T) and (b) condition factor (K) of parental *Micropterus dolomieu* across sampled lakes (see Fig. 1). Sample sizes are shown on individual bars. Dissimilar uppercase letters denote significant differences between means (Tukey's *post hoc* test, $P < 0.05$).

ENZYME ACTIVITY AND PROTEIN CONCENTRATION

NDPK in parental males differed between lakes and the offspring developmental period [Table I and Fig. 7(a)]. NDPK activities were the highest for southern fish, followed by mid-northern latitude fish and lowest for northern fish. NDPK also generally decreased from the egg to the free-swimming fry stage [Table I and Fig. 7(a)]. P_M did not differ between lakes or between the offspring development stage overall, but did differ between offspring development stage within lakes [Table I and Fig. 7(b)]. All means \pm s.e. of the physiological sampling are presented in Table II for both nest-guarding stages.

TABLE I. Comparison of physiological indices of parental *Micropterus dolomieu* in five lakes across their latitudinal range (see Fig. 1) and the offspring development stage. Lake refers to locations where fish were collected and has five levels: Bull Shoals Lake, MO–AR, U.S.A. (36° 25' N; 92° 45' W), Table Rock Lake, MO, U.S.A. (36° 35' N; 93° 50' W), Big Rideau Lake Ontario, Canada (44° 46' N; 76° 13' W), Charleston Lake (44° 32' N; 76° 00' W), and Rainy Lake, Ontario, Canada (48° 38' N; 93° 15' W). Stage refers to the development stage of offspring (egg and free-swimming fry)

Response	Source	d.f.	F ratio	P-value
I_G	Lake	4	25.9	<0.001
	Stage	1	35.0	<0.001
	Interaction	4	6.0	<0.001
I_H	Lake	4	2.1	<0.01
	Stage	1	5.3	<0.05
	Interaction	4	0.4	>0.05
Eviscerated body lipid (g kg ⁻¹)	Lake	4	14.4	<0.001
	Stage	1	0.2	>0.05
	Interaction	4	1.7	>0.05
Body energy density (MJ kg ⁻¹)	Lake	4	4.9	<0.01
	Stage	1	0.0	>0.05
	Interaction	4	1.1	>0.05
Liver lipid (g kg ⁻¹)	Lake	4	11.4	<0.001
	Stage	1	0.2	>0.05
	Interaction	4	1.0	>0.05
Stomach content wet mass (g)	Lake	4	6.2	<0.001
	Stage	1	0.1	>0.05
	Interaction	4	1.7	>0.05
Plasma protein (g l ⁻¹)	Lake	2	12.5	<0.001
	Stage	1	2.3	>0.05
	Interaction	2	0.3	>0.05
Triglycerides (mmol l ⁻¹)	Lake	2	3.4	<0.05
	Stage	1	1.8	>0.05
	Interaction	2	0.8	>0.05
Cholesterol (mmol l ⁻¹)	Lake	2	6.1	<0.01
	Stage	1	3.7	>0.05
	Interaction	2	0.1	>0.05
Na (mmol l ⁻¹)	Lake	2	3.6	<0.05
	Stage	1	9.0	<0.01
	Interaction	2	6.0	<0.01
Cl (mmol l ⁻¹)	Lake	2	2.9	>0.05
	Stage	1	3.6	>0.05
	Interaction	2	2.1	>0.05
Glucose (mmol l ⁻¹)	Lake	2	2.7	>0.05
	Stage	1	3.0	>0.05
	Interaction	2	2.2	>0.05
NDPK activity (IU mg protein ⁻¹)	Lake	2	6.3	<0.001
	Stage	1	14.0	<0.05
	Interaction	2	2.4	>0.05
Axial muscle protein concentration (mg l ⁻¹)	Lake	2	5.5	>0.05
	Stage	1	0.1	>0.05
	Interaction	2	4.7	<0.01

I_G , gonado-somatic index; I_H , hepato-somatic index; NDPK, nucleoside diphosphate kinase.

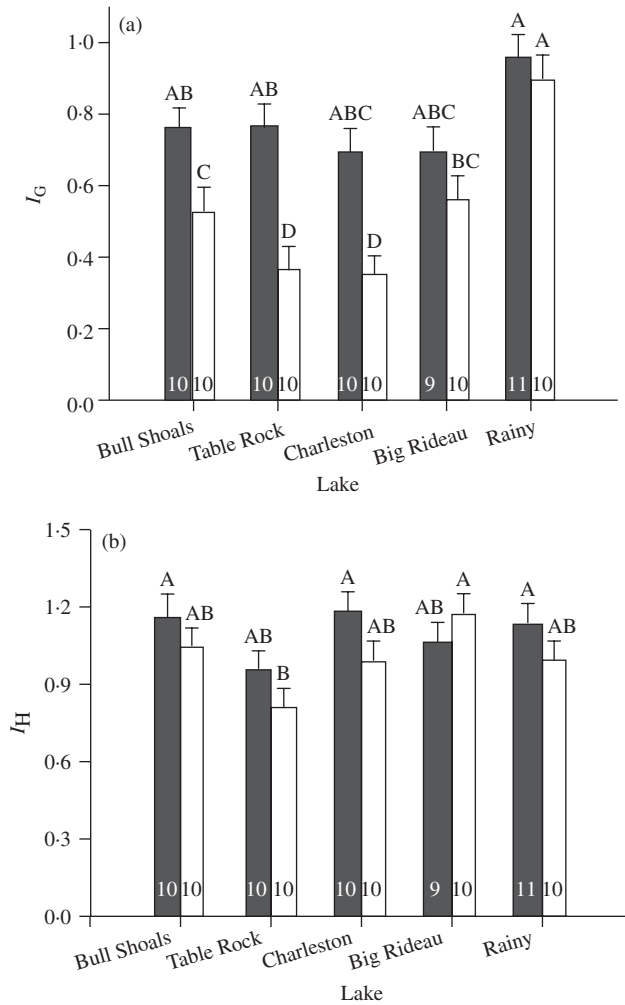


FIG. 3. Mean \pm S.E. (a) gonado-somatic (I_G) and (b) hepato-somatic (I_H) indices of parental *Micropterus dolomieu* guarding eggs (■) and free-swimming fry (□) across lakes at different latitudes (see Fig. 1). Sample sizes are shown on individual bars and dissimilar uppercase letters denote significant differences between means (Tukey's *post hoc* test, $P < 0.05$).

DISCUSSION

This study examined the comparative energetics and physiology of parental care in *M. dolomieu* in five different lakes. Since these lakes are distributed across a broad latitudinal gradient, the sample size is rather restricted. The interpretation of this study is limited by the distribution of the sampling sites, where the distance between the southern site latitude (8° , *c.* 900 km north–south) and mid-northern latitude site is greater than the distance from the mid-northern latitude site to the most-northern site (4° latitude, *c.* 450 km north–south). Finally, given the large amount of effort required to sample multiple lakes within a single spawning season to control for annual variation meant that only one or two lakes were sampled at

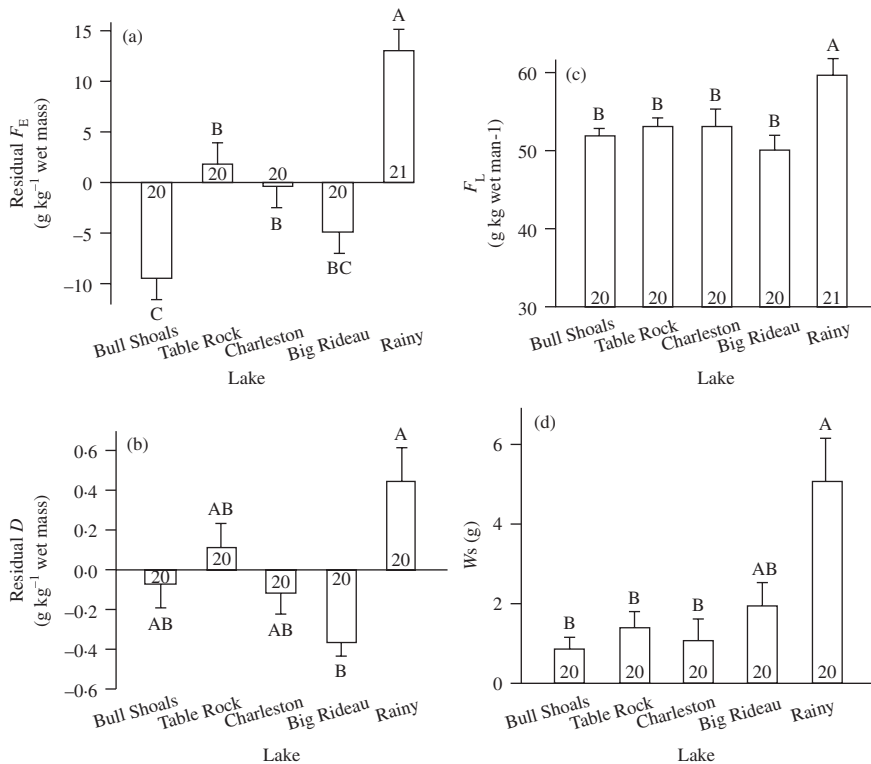


FIG. 4. Mean + s.e. residual (a) whole-body lipid content (F_E) and (b) energy density (D) of eviscerated body (excludes liver, stomach and gonads), (c) liver lipid (F_L) and (d) stomach contents mass (M_S) of parental *Micropterus dolomieu* during the parental care period across lakes at different latitudes (see Fig. 1). Males guarding eggs and free-swimming fry have been pooled. Sample sizes are shown on individual bars and dissimilar uppercase letters denote significant differences between means (Tukey's *post hoc* test, $P < 0.05$).

each latitude. Hence, without more replicates at each latitude, care must be taken with interpretation given that a single lake with unique characteristics could alter the findings. Nonetheless, these data provide an opportunity to explore how the costs of parental care vary relative to broad-scale environmental variation and will serve as a basis for future experimentation and hypothesis testing. Although lakes are presented separately in the statistical analysis, results are discussed within the framework of latitude. Overall, latitude influenced the energy status of parental males, the trade-offs between reproduction and growth, but did not influence indicators of chronic stress nor the magnitude of change of energetic or physiological indicators across the parental care period.

COMPARISON OF INITIAL PARENTAL ENERGY STORES AND INDICES ACROSS LATITUDES

Environmental variation, in the form of broad-scale latitudinal differences, had a significant effect on the energy status and physiological state of parental care-providing *M. dolomieu*. Northern *M. dolomieu* had significantly higher condition,

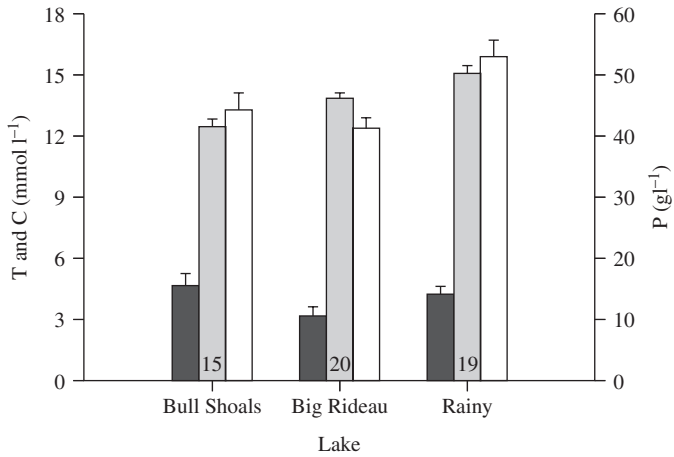


FIG. 5. Mean + s.e. plasma triglycerides (T; ■), protein (P; □) and cholesterol (C; □) of parental *Micropterus dolomieu* between lakes at different latitudes (see Fig. 1). Sample sizes are identical for each physiological variable and are shown on individual bars.

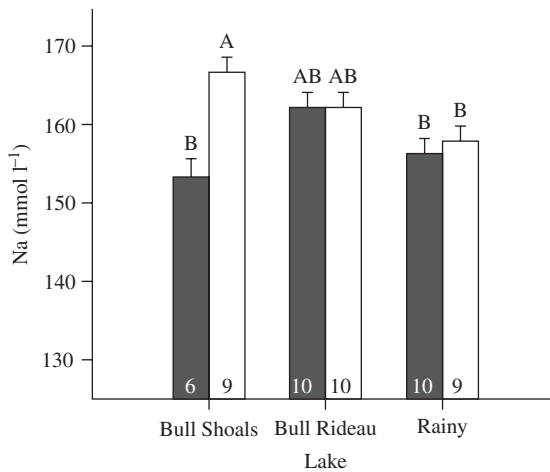


FIG. 6. Mean + s.e. plasma sodium (Na) of parental *Micropterus dolomieu* guarding eggs (■) and free-swimming fry (□) across lakes at different latitudes (see Fig. 1). Sample sizes are shown on bars and dissimilar uppercase letters denote significant differences between means within a given plasma indicator (Tukey's *post hoc* test, $P < 0.05$).

energy reserves and indicators of nutritional status than southern males. This result is consistent with modelling studies conducted by Shuter *et al.* (1980) and Garvey & Marschall (2003) which predict that males in northern clines need to (1) invest more energy in lipids and gonads, typically by autumn of the previous year and (2) be more selective about investing energy into reproduction (*i.e.* take 'reproductive holidays'). The alternative hypothesis, which predicted that fish in northern clines may begin parental care in poorer physiological condition, given the harsher winters, is refuted,

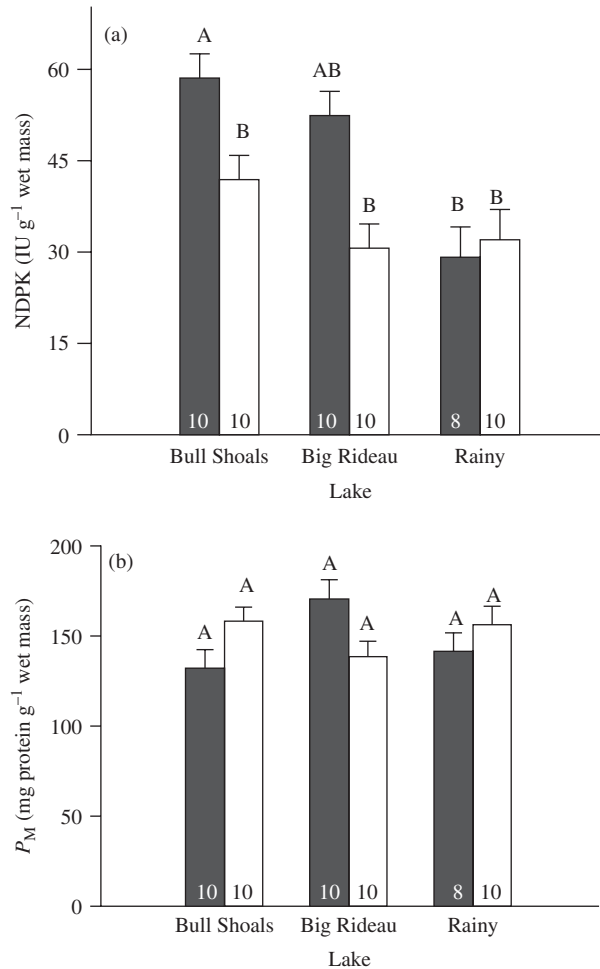


FIG. 7. Mean + s.e. (a) nucleoside diphosphate kinase (NDPK) activity and (b) axial muscle protein concentration (P_M) of parental *Micropterus dolomieu* guarding eggs (■) and free-swimming fry (□) across lakes at different latitudes (see Fig. 1). Sample sizes are shown on individual bars and dissimilar uppercase letters denote significant differences (Tukey's *post hoc* test, $P < 0.05$).

which is most likely related to other life-history decisions. It is generally accepted that individuals that encounter size-selective mortality (*e.g.* size-related resistance to winter starvation) tend to invest heavily in growth and delay reproduction (Shuter *et al.*, 1980). In systems where reproductive success is related to body size and energy intake is limited due to a short growing season, it would be advantageous to first invest in body size and delay reproduction (Garvey & Marschall, 2003). Many North American and European freshwater species follow this pattern and show evidence for latitudinal differences in growth, size at maturation and longevity (Belk & Houston, 2002; Blanck & Lamouroux, 2007), where northern fishes grow more slowly, mature later and have longer life spans than southern conspecifics. Bergman's rule states that intraspecific variation in body size is common and that

body size typically increases with latitude (Mayr, 1956). This theory is generally supported for endotherms (Ashton *et al.*, 2000) but has only recently been tested on freshwater fishes (Belk & Houston, 2002). Generally, large-bodied North American freshwater species show decreases in body size with latitude with two exceptions: *M. dolomieu* and pike *Esox lucius* L. These species showed a positive trend between body size and latitude due to a significant positive correlation between longevity and latitude (Belk & Houston, 2002). As such, northern *M. dolomieu* may be older and larger, which enables them to better accumulate stores through simple allometric functions; larger individuals have exponentially more energy reserves than smaller individuals (Schmidt-Nielsen, 1984).

The higher energy density of fish in northern clines was explained in part by the higher incidence of feeding in northern males during the care period as revealed by greater stomach-content masses. In addition, fish from the most northerly latitude had higher plasma concentrations of protein and cholesterol, indicating that males had not been fasting (Wagner & Congleton, 2004). Although it is generally considered that *M. dolomieu* have limited opportunity to forage while providing parental care (Hinch & Collins, 1991; Mackereth *et al.*, 1999), this study has shown that foraging opportunities or incidences are site specific. While stomach-content masses are generally lower during the parental care period relative to normal foraging activities (Mackereth *et al.*, 1999), northern fish in this study clearly consumed more food during this period than southern and mid-northern latitude fish. This is the first evidence of site-specific foraging activity during *M. dolomieu* parental care, but it alone may not fully explain the two and three-fold difference in whole-body energy stores and energy densities between fish sampled from different latitudes.

In addition, *M. dolomieu* are known to reduce overall activity and foraging when water temperatures are $<10^{\circ}\text{C}$ (Kolok, 1991), which is accompanied by lower metabolic demands (Adams *et al.*, 1982). During the winter months lakes at mid-northern and northern latitudes are ice-covered and winter water temperatures will generally approach 4°C . Lakes at southern latitudes will show more variation in temperature during the winter months and will generally be closer to mean air temperature (between 6 and 10°C ; NOAA). Although it is generally well accepted that all populations reduce foraging and activity when water temperatures are $<10^{\circ}\text{C}$, the metabolic costs of maintenance in southern clines could be more elevated than northern clines due to the relationship between metabolic costs and temperature. Thus, southern fish may have lower energy stores and densities due to higher metabolic costs associated with a slightly warmer winter.

Although these possibilities would explain the energetic differences between the most southern population and the most northern population, they do not clarify the discrepancy between mid-northern and northern-latitude males. Here, mid-northern latitude males of similar size to northern males have lower I_G , residual whole-body lipid stores, whole-body energy densities and liver-lipid content. Differences between these groups could alternatively be explained by a difference in food availability or by the presence and absence of 'reproductive holidays'. At mid-northern latitudes there is evidence that a proportion of male *M. dolomieu* spawn only once in their lifetime and are functionally semelparous (Raffetto *et al.*, 1990; Barthel *et al.*, 2008), whereas other individuals spawn consistently throughout their adult life or spawn inconsistently during adulthood (Barthel *et al.*, 2008). Although this annual variation in reproductive contribution remains mostly unexplained, it is suggested that the

energetically costly nature of parental care may inhibit males from performing this activity every year (Ridgway & Friesen, 1992). If energy availability explains these patterns of reproductive contribution, latitudinal variation in these patterns is also possible. Latitudinal variation in reproductive effort has been reported for a number of fish species, e.g. perch *Perca fluviatilis* L. (Heibo *et al.*, 2005) and American shad *Alosa sapidissima* (Wilson) (Leggett & Carscadden, 1978). In some cases, individuals in northern populations spawn repeatedly, whereas their southern counterparts spawn only once (Leggett & Carscadden, 1978). In *P. fluviatilis*, reproductive investment decreased with latitude (Heibo *et al.*, 2005). Although fish from the northern lake invested more into reproduction (I_G), the reproductive background of the individuals in this study is unknown. It is possible that this comparison includes individuals which have spawned more than once since maturity and individuals which are spawning for the first time or after more than one season of reproductive absence. With the data currently available from *M. dolomieu*, it is impossible to determine which populations show higher proportions of functional semelparity.

In addition, seasonal variation in energy stores has been shown at mid-northern latitudes for *M. dolomieu* (Mackereth *et al.*, 1999). In this study, male *M. dolomieu* were found to build large lipid stores in the autumn, which then decreased over winter and did not recover in the spring before the reproductive period. It is unclear whether fish were investing energy in I_G or were simply unable to regain energy stores at the start of the warmer season. If this seasonal variation is not consistent across latitudes, it is possible that northern fish have the ability to increase stores in the spring immediately prior to reproduction or forage during the winter and that this contributes to their higher lipid stores at the onset of reproduction.

CHANGES IN ENERGETIC AND PHYSIOLOGICAL STATUS OF PARENTAL MALES DURING PARENTAL CARE ACROSS LATITUDES

Overall there was no difference across latitudes in the magnitude of change in energetic and physiological status of males providing parental care. The measures of direct energy loss, such as F_E , F_L and indicators of nutritional status, showed no interaction effects, which indicates that the effect of the offspring development stage was similar across latitudes. More specifically, this study generally noted no effect of the offspring development stage. Although the data support the prediction related to the magnitude of change in energetic and physiological variables of parental males, there was a surprising lack of difference in physiological variables between the offspring development stages. As mentioned above, parental care activities have been shown to be relatively costly in many systems (Townsend, 1986; Moreno, 1989; Coleman & Fischer, 1991) including for *M. dolomieu* (Gillooly & Baylis, 1999; Mackereth *et al.*, 1999). In general, fish were not in poorer physiological or energetic condition near the end of parental care. One possibility may be that the physiological tools used in this study may be unable to detect other forms of ecological costs that these individuals may be incurring such as reduced future reproductive opportunities (Coleman *et al.*, 1985). For instance, in some systems, a large proportion of males provide parental care only once in their lives and are functionally semelparous (Barthel *et al.*, 2008) and mortality (reproduction-related and reproduction-independent) can be as high as 94% following first reproduction

(Wiegmann & Baylis, 1995), both of which would suggest that parental care is not cost free.

I_G , I_H and Na were physiological variables that differed across the parental care period. Male I_G decreased from the egg stage to the free-swimming fry stage for southern and mid-northern latitude fish, whereas northern fish had higher I_G values that were maintained throughout the care period. The ability of northern fish to maintain high I_G most likely relates to their superior condition as indicated by whole-body energy stores and condition factor. The decrease in male I_G across the parental care period for mid-northern and southern latitudes is consistent with other work that showed I_G decreases across the parental care period most probably due to the mobilization of energy stores and expression of sperm during spawning (Mackereth *et al.*, 1999). In addition, male I_H typically decreased from the egg stage to the free-swimming fry stage. Some energy may be stored as the carbohydrate glycogen, and a substantial amount of lipid is stored in muscle due to absolute volume of muscle relative to liver. Liver lipid stores are still one of the main forms of energy stores and enables rapid energy mobilization for many species of fishes (Love, 1970; including *M. dolomieu*, Mackereth *et al.*, 1999), and the loss of liver mass is evidence that parental care is costly.

Na was the only stress-indicator which differed between lakes. Na increased during parental care in fish from the south, whereas it remained relatively constant at mid-northern and northern latitudes (Table II). Interestingly, the significant increase in Na levels in parental males with offspring from the egg to the free-swimming fry stage at the southern latitude is inconsistent with other studies. Wagner & Congleton, (2004) found that Na levels decrease as a secondary stress response, *i.e.* fish typically lose ions in fresh water when exposed to stress (Wendelaar Bonga, 1997; Barton *et al.*, 2002). There may be other ecological factors driving the differences in Na (*e.g.* differential food sources). Alternatively, Na may not be as good an indicator of chronic stress in centrarchids (Hanson & Cooke, 2009), as it is for salmonids (Wagner & Congleton, 2004) since no other stress-induced variable (cholesterol and glucose) differed between latitudes.

TRADE-OFFS BETWEEN GROWTH AND REPRODUCTION

The maintenance of capacity for growth was influenced by latitude as well as offspring development stage. As predicted, northern fish showed lower biosynthetic capacity, followed by mid-northern latitude fish and finally southern latitude fish. This, coupled with their lower body condition, demonstrates that southern fish are able to upregulate NDPK and maintain growth potential relative to mid-northern and northern-latitude males. These results show support for models developed for largemouth bass *Micropterus salmoides* (Lacépède) which attempt to maximize expected lifetime fitness through differential investment into growth or reproduction across latitudes (Garvey & Marschall, 2003). Although northern and southern fish invested similarly into growth at low rations, southern fish were able to invest into growth and reproduction at high rations, whereas northern fish at high rations varied their investment seasonally: growth in the summer, lipid reserves during the autumn and finally allocation of energy to reproduction early in the year (Garvey & Marschall, 2003). This is consistent with the results for *M. dolomieu* from this study since southern and mid-northern fish showed decreased NDPK from the egg stage to

TABLE II. Physiological variables (mean \pm s.e.) measured in nesting male *Micropterus dolomieu*. Fish were sampled ($n = 10$) at two nest-guarding stage (egg and free-swimming fry) from five lakes [Bull Shoals Lake (BS), Table Rock Lake (TR), Charleston Lake (Char), Big Rideau Lake (BR) and Rainy Lake (RL)] across 12° of latitude (see Fig. 1). Lakes are presented in order of increasing latitude

Variables	Egg					Free-swimming fry				
	BS	TR	Char	BR	RL	BS	TR	Char	BR	RL
I_G	0.76 \pm 0.03	0.77 \pm 0.08	0.69 \pm 0.07	0.70 \pm 0.05	0.96 \pm 0.10	0.5 \pm 0.04	0.4 \pm 0.04	0.4 \pm 0.01	0.56 \pm 0.04	0.9 \pm 0.05
I_H	1.15 \pm 0.10	0.95 \pm 0.09	1.17 \pm 0.06	1.05 \pm 0.06	1.13 \pm 0.05	1.03 \pm 0.06	0.80 \pm 0.04	0.97 \pm 0.05	1.15 \pm 0.05	0.98 \pm 0.08
Eviscerated body lipid content (g kg ⁻¹)	23.9 \pm 2.3	32.5 \pm 2.5	41.2 \pm 2.5	36.4 \pm 1.6	57.9 \pm 2.3	24.5 \pm 2.3	34.5 \pm 4.2	39.8 \pm 5.1	31.6 \pm 3.3	55.5 \pm 3.7
Eviscerated body water content (g kg ⁻¹)	736.1 \pm 4.1	723.3 \pm 8.1	735.7 \pm 3.5	733.6 \pm 4.3	706.3 \pm 3.5	716.9 \pm 8.5	730.4 \pm 8.3	736.8 \pm 7.6	744.1 \pm 4.1	711.8 \pm 4.9
Eviscerated body ash content (g kg ⁻¹)	35.0 \pm 2.5	37.4 \pm 2.8	34.5 \pm 3.3	43.8 \pm 3.4	40.9 \pm 2.9	34.6 \pm 2.1	33.9 \pm 3.9	29.8 \pm 2.9	34.4 \pm 2.9	39.1 \pm 2.0
Eviscerated body protein content (g kg ⁻¹)	204.9 \pm 11.3	206.8 \pm 24.7	188.5 \pm 14.7	186.2 \pm 9.9	194.8 \pm 19.3	223.8 \pm 6.2	202.3 \pm 7.2	193.7 \pm 3.3	189.8 \pm 3.3	193.5 \pm 4.3
Eviscerated body energy density (MJ kg ⁻¹)	5.0 \pm 1.3	5.3 \pm 1.8	5.3 \pm 0.1	5.1 \pm 0.1	6.0 \pm 0.1	5.4 \pm 0.2	5.3 \pm 0.2	5.3 \pm 0.2	5.0 \pm 0.1	5.9 \pm 0.1
Liver lipid (g kg ⁻¹)	49.8 \pm 1.3	53.3 \pm 2.2	52.8 \pm 0.9	50.6 \pm 0.7	60.1 \pm 1.2	53.9 \pm 1.8	53.1 \pm 1.9	53.3 \pm 2.2	49.6 \pm 1.2	59 \pm 1.4
Stomach content wet mass (g)	0.89 \pm 0.34	0.93 \pm 0.34	0.51 \pm 0.33	1.27 \pm 0.23	6.50 \pm 1.64	0.87 \pm 0.53	1.80 \pm 0.62	1.60 \pm 1.10	2.64 \pm 1.11	3.65 \pm 1.42
Plasma protein (g l ⁻¹)	40.0 \pm 1.2	na	na	45.0 \pm 1.3	50.0 \pm 2.2	43.1 \pm 1.1	na	na	47.4 \pm 2.1	50.4 \pm 1.7
Triglycerides (mmol l ⁻¹)	4.7 \pm 0.7	na	na	2.7 \pm 0.2	3.4 \pm 0.4	4.7 \pm 0.7	na	na	3.8 \pm 0.7	5.3 \pm 0.7
Cholesterol (mmol l ⁻¹)	12.3 \pm 1.3	na	na	11.8 \pm 0.5	14.8 \pm 1.4	14.0 \pm 1.0	na	na	13.1 \pm 0.8	16.6 \pm 1.0
Na (mmol l ⁻¹)	153.2 \pm 2.7	na	na	162.3 \pm 2.2	156.2 \pm 1.2	166.7 \pm 2.5	na	na	162.1 \pm 2.2	157.9 \pm 1.2
Cl (mmol l ⁻¹)	111.3 \pm 2.2	na	na	121.5 \pm 2.0	111.2 \pm 1.8	112.2 \pm 9.9	na	na	110.5 \pm 3.4	107.6 \pm 3.7
Glucose (mmol l ⁻¹)	4.2 \pm 0.6	na	na	3.4 \pm 0.2	3.3 \pm 0.3	3.3 \pm 0.2	na	na	2.7 \pm 0.2	3.5 \pm 0.3
NDPK activity (IU g ⁻¹ wet mass)	58.2 \pm 3.5	na	na	52.3 \pm 7.8	29.1 \pm 7.0	41.7 \pm 4.3	na	na	30.3 \pm 2.4	31.9 \pm 2.2
Axial muscle protein concentration (mg protein g ⁻¹ wet mass)	131.4 \pm 10.0	na	na	171.0 \pm 6.4	141.0 \pm 7.6	156.6 \pm 13.2	na	na	138.0 \pm 6.6	156.0 \pm 11.9

I_G , gonado-somatic index; I_H , hepato-somatic index; NDPK, nucleoside diphosphate kinase; na, not collected.

the free-swimming fry stage, whereas northern latitude fish had significantly lower capacity for biosynthetic capacity throughout parental care.

Environmental factors had significant effects on the energy status and physiological state of care-providing *M. dolomieu* and showed evidence of affecting the costs of parental care across latitudes. Latitude also influenced how parental *M. dolomieu* balanced the trade-off between growth and reproduction during the parental care period. Northern *M. dolomieu* were larger, had higher length-corrected energy stores and indicators of nutritional status, but showed trade-offs between growth and reproduction during the parental care period. This study highlights how climatic variation may affect parental investment and the cost of reproduction across a broad environmental scale. This study also shows that physiological indicators provide useful information to help elucidate the costs and consequences of reproduction and complex behaviours such as parental care in wild fishes and have the potential to increase the mechanistic understanding of critical life-history trade-offs, the evolution of parental care and parental care decisions (Altmann & Altmann, 2003).

We would like to thank M. Savoie and R. Cunjak from the University of New Brunswick for their assistance in setting up the lipid extractions. We would like to thank N. Garceau, K. Murchie, L. Thompson, T. Redpath, S. Wilson and C. Yick for their help in the laboratory. We would also like to thank M. Caputo, A. Gingerich, K. Hanson, J. Koppelman, S. Landsman, D. McLeod, C. O'Connor, A. O'Toole, T. Redpath, M. Sieper and R. Sunder for their outstanding help in the field. Additional field support was provided by the Ontario Ministry of Natural Resources (Fort Frances office) and the Missouri Department of Conservation. Several anonymous reviewers kindly provided comments on earlier versions of this manuscript. Research activities were supported by NSERC Discovery Grants to S.J.C. and P.C. and the Rainy Lake Charity Fisheries Trust.

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