



Oxygen consumption in relation to current velocity and morphology in the highly invasive crayfish *Procambarus clarkii*

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Abstract The red swamp crayfish (*Procambarus clarkii*) is the most widely spread freshwater crayfish worldwide. Competing physiological traits can influence invasion success in any given environment by limiting the available scope for aerobically demanding activities. While high flows have been associated with reduced crayfish movement upstream, the effects of flow alteration on their metabolic demands have been largely overlooked. In this study, we estimated routine metabolic rate (RMR) at rest and oxygen consumption rates of crayfish under different current velocities in a flume respirometer, while maximum metabolic rate (MMR) was determined using the exhaustive chase protocol. We also measured some morphometric variables in males and females of crayfish. Oxygen uptake substantially increased with crayfish size and current velocity due to increased energy

expenditure to overcome drag and hold a stationary position. Sexual dimorphism in morphological traits did not lead to sexual differences in oxygen uptake. Moreover, we found that individuals operated close to their maximum aerobic capacity at elevated current velocities ($\geq 25 \text{ cm s}^{-1}$). This suggested that the high flow-driven energetic demand may compromise the energy available for reproduction, growth and dispersal, thereby affecting overall fitness. These metabolic constraints could partly explain the failed invasions of invasive crayfish in fast-flowing waters.

Keywords Metabolic rates · Water flow · Morphological traits · Invasive species · Cambaridae

Introduction

The introduction of species on a global scale is an increasing trend and it represents one of the principal threats for biodiversity. In fact, invasive species are the second highest contributor to species extinction after the destruction of habitats (IUCN, 2020). Half of the total invasive species in the Iberian Peninsula are fish and crayfish (Vedia & Miranda, 2013) and they have the potential to impact the native biota and ecosystems (Lodge et al., 2000; Maceda-Veiga et al., 2013; Vedia & Miranda, 2013). Previous studies have demonstrated that some abiotic factors such as streamflow and habitat conditions can have strong influences on crayfish populations (Rallo

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& García-Arberas, 2002; Light, 2003; Bubb et al., 2004; Cruz & Rebelo, 2007). While in regulated streams low flows favored the spread of invasive crayfish (Chester et al., 2014), high flows (e.g., seasonal floods) reduced upstream dispersal and movements (Gamradt & Kats, 1996; Bernardo et al., 2003; Light, 2003). In addition, Kerby et al. (2005) showed that the combination of high flow velocity and large instream barriers (i.e., waterfalls, culverts) increased the spread of crayfish downstream but largely restricted their movement to upstream stretches. Thus, management of flow regimes to include bankfull or greater flows may be a useful tool to control invasive crayfish in streams (Bernardo et al., 2003; Maceda-Veiga et al., 2013; Chester et al., 2014).

Ecophysiological features have a fundamental role in shaping the distribution and abundance of riverine species (Somero, 2005), and the study of oxygen consumption rates has received growing attention to understand plastic and evolutionary responses to environmental change (e.g., Marras et al., 2015). To determine the respiratory capacity of an animal several important metabolic traits can be measured depending on the activity level. The standard metabolic rate (SMR) represents the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (Chabot et al., 2016). Thus, it is an integrated measure of the physiological energy expenditures involved in the anabolism and catabolism of tissues and organism homeostasis (Fry, 1971). The related term routine metabolic rate (RMR) refers to the average rate of metabolism when the animal is undergoing normal behaviors or spontaneous activity (Metcalf et al., 2016). Another important metabolic trait is the maximum metabolic rate (MMR), which is defined as the maximum rate of oxygen uptake that an animal can achieve to create ATP aerobically (Norin & Clark, 2016). Therefore, the routine metabolic rate falls somewhere between standard and maximum metabolic rate and have important implications for ecologists interested in energy expenditure of animals in the field (cf. Steffensen, 2005). The difference between the lower and upper limits of oxygen consumption rates is called aerobic scope (AS) and represents an animal's capacity to simultaneously supply oxygen to energy-demanding fitness traits such as activity, reproduction, and growth (Clark et al., 2013). Given that an individual must operate within the confines of its aerobic scope, a reduction in scope could

lead to a reduction in its performance (e.g., reduced growth rates in fish; Leal et al., 2021), and potentially overall fitness (Fry, 1947; Boddington, 1978; Claireaux & Lefrançois, 2007).

Both body size and shape are important intrinsic parameters affecting hydrodynamics and oxygen consumption rates in aquatic organisms (Ohlberger et al., 2016; Rubio-Gracia et al., 2020a,b) and, in particular, body shape is an important factor for energy-reducing strategies (Webb, 1993). A few number of studies have previously investigated the relative importance of some morphological characteristics of crayfish to overcome drag forces and maintain position against water flow. The relationship between the drag on crayfish and current speed has been well established, in which larger individuals (measured in carapace length) experience higher drag forces (Maude & Williams, 1983). Related to sexual differences, males typically experience greater drag than females due to the proportionately larger size of the males' claw, the so-called 'chela' (Maude & Williams, 1983; Tullis & Straube, 2017). Regarding body shape, lengths of carapace height and carapace width in crayfish determine the shape of the cephalothorax. It has been shown that crayfishes can maintain station at elevated water current velocities by lowering their cephalothorax and abdomen down in a streamlined posture, thus decreasing the effect of drag (Maude & Williams, 1983).

The red swamp crayfish *Procambarus clarkii* (Girard, 1852) is a species of decapod of the family Cambaridae. It is native to the wetlands of the southern United States of America (U.S.A) and north-eastern Mexico, but nowadays this species is found in Africa, Asia, Europe, and North and South America, thus becoming the most invasive crayfish species in the world (Vedia & Miranda, 2013). The invasion success of the red swamp crayfish has been mainly attributed to its broad environmental tolerance, high fecundity, rapid growth, and high aggressiveness, among other factors (e.g., see Geiger et al., 2005). With regard to its broad environmental tolerance, the red swamp crayfish can tolerate sudden increases in flow and intervals of drought, these being common ecological conditions in Mediterranean wetlands (Geiger et al., 2005). In streams, however, they are mainly found in slow-moving waters and pools (Anastácio & Marques, 1998; Kerby et al. 2005; Dörr et al.,

2006; Gherardi, 2006; Maceda-Veiga et al., 2013; van Kuijk et al., 2021). Whereas some research has identified current velocity as a key factor limiting crayfish distribution (Gil-Sánchez & Alba-Tercedor, 2002; Cruz & Rebelo, 2007; Maceda-Veiga et al., 2013), almost no information is available regarding the metabolic demands of the red swamp crayfish at different current velocities.

In this study, we investigated the oxygen consumption rate of the red swamp crayfish across an ecologically relevant range of current velocities (i.e., 0, 10, 25, 35, and 50 cm s⁻¹). It was hypothesized that higher current velocities would result in increased oxygen uptake (H1), and that the highest rate of oxygen consumption measured at high flows would represent the upper boundary of oxygen uptake in crayfish (H2), given that this invasive species could be sensitive to direct flow exposure (Gil-Sánchez & Alba-Tercedor, 2002). Moreover, intra-specific variation in oxygen uptake would be determined by differences in body size and morphological features of the individuals (H3), e.g., due to sexual dimorphism (Tullis & Straube, 2017). The resulting knowledge from this study can provide mechanistic understanding of the metabolic constraints of the invasive crayfish to cope with higher water flows.

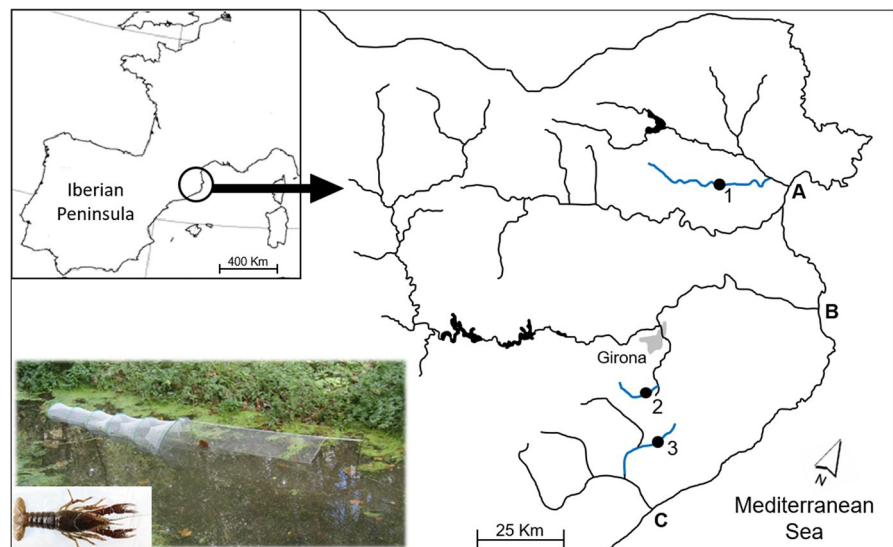
Methods

Animal collection and holding conditions

In October 2018, 30 red swamp crayfish were caught from the Manol stream (Muga river basin), Santa Maria stream (Tordera river basin), and Riudevila stream (Ter river basin) in the northeast of Spain (Fig. 1). The crayfish sampling was conducted in different streams in order to collect a sufficient number of individuals for the study. These three streams have similar discharge during the year and therefore the different freshwater crayfish populations were expected to experience relatively similar water flows. According to data from the Catalan Water Agency, the water flow velocities of these streams between 2005 and 2018 were 20 ± 10 cm s⁻¹ (average ± standard error) for Manol stream, 31 ± 24 cm s⁻¹ for Santa Maria stream and 38 ± 20 cm s⁻¹ for Riudevila stream. Individuals were caught using fyke nets, which are long cylindrical netting bags usually with several netting cones fitted inside the netting cylinder to make entry easy and exit difficult. These nets were fixed on the stream bottom by steel bars (Fig. 1).

Crayfish were transported to the housing facility at the University of Girona where they were separated by sex on the basis of the male gonopods and the female gonopores. Individuals were randomly held in groups of 4 individuals of the same sex in 40 l aquariums, except for larger males which were

Fig. 1 Map showing the three sampling sites (black circles) in the NE of Iberian peninsula (Spain): 1. Manol stream (A, Muga river basin), 2. Riudevila stream (B, Ter river basin), and 3. Santa Maria stream (C, Tordera river basin). Crayfish were caught using the type of fyke net shown in the image. The streams are highlighted in blue and the main city (Girona) and reservoirs in the surroundings are represented by shaded areas



kept alone to avoid aggressive behaviors toward conspecifics. Aquariums were filled with dechlorinated tap water (conductivity $\sim 320 \mu\text{S cm}^{-1}$, $\text{pH} \sim 7.6$), at natural photoperiod conditions and at a temperature of $18 \pm 1 \text{ }^\circ\text{C}$, being within the range ($15\text{--}25 \text{ }^\circ\text{C}$) of normal environmental temperatures experienced by crayfish (Espina et al., 1993). Additionally, aquariums contained gravel substrate and artificial hides (i.e., PVC pipe sections) and were supplied with recirculated, filtered freshwater (particle filtered and ozone sterilized), and vigorous aeration. Water changes of 30% of the total volume were conducted twice a week in each aquarium to assist with maintaining water quality. Crayfish were allowed two weeks to habituate to captive conditions prior to experimentation. Crayfish were fed ad libitum once a day with commercial shrimp pellets and bloodworms (*Chironomus* spp.). Feeding ceased 48 h before the respirometry trials to ensure that oxygen consumption measurements were not affected by the digestion, absorption, and assimilation of the meal (Clark et al., 2010; Chabot et al., 2016). Those individuals starting to moult were transferred into an individual aquarium without feed and were not used for respirometry tests until the carapace hardened, because ecdysis may substantially increase oxygen consumption rates (Rice & Armitage, 1974). No mortalities occurred during acclimation period. Experiments were conducted between autumn and early spring to avoid the breeding season of the species. At the end of the experiments, all individuals were euthanized according to a standardized protocol using concentrated MS222 in an aerated-water holding tank. Animal research under captive conditions and the fieldwork were authorized by the Autonomous Government of Catalonia (SF/1089/2017) and the Commission of Animal Experimentation (Ref.: CEA-OH/9673/1).

Respirometry

To measure oxygen consumption rates (MO_2) we used a Blazka-type flume respirometer (Loligo® Systems, Viborg, Denmark), which consisted in a rectangular measuring recirculation system equipped with a swim chamber of 5 l (30 cm length \times 7.5 cm height \times 7.5 cm width). Given that crayfish were not expected to swim against water flow as fish do, we placed a rectangular piece of slate (25 cm long \times 7 cm wide \times 0.6 cm high) at the

bottom of the flume respirometer to provide a wrinkled layer. This wrinkled layer likely increased the boundary layer (i.e., a pocket of low-velocity water) inside the flume respirometer, especially for smaller individuals. However, it allowed crayfish to grip against the water current, and thus we could test the effects of water flow on crayfish metabolic demands. A propeller connected to the motor outside of the flume respirometer generated a continuous laminar flow, which was made rectilinear by placing a honeycomb plastic screen at the entrance of the flume respirometer. For calibration purposes, water flow velocities were measured in the center of the flume and above the substratum using a handheld digital flow meter (Höntzch, 4–20 mA/0–10 V input). The flume respirometer was immersed in an external water bath of 25 L, and an automated Eheim pump flushed aerated water at a rate of 10 l min^{-1} between flush cycles. Additionally, we connected the external water bath to a supply plastic tank (300 L) that continuously provided air-saturated water to the water bath, and then water was recirculated again through a decantation system. The supply tank was equipped with an automated liquid cooler (85 W, 972.46 BTU/h, J.P Selecta®) that allowed us to maintain temperature at $18 \pm 1 \text{ }^\circ\text{C}$.

We used an optical fiber instrument (Witrox 1; Loligo® Systems, Tjele, Denmark) to determine dissolved oxygen concentration in the water ($\text{mg O}_2 \text{ l}^{-1}$). A temperature probe (Pt1000 temperature sensor; Witrox 1; Loligo® Systems, Tjele, Denmark) was also used for the automated compensation of oxygen data to changes in temperature and barometric pressure in real time. Rates of oxygen consumption were measured using computerized, intermittent-flow respirometry. We used repeated respirometric loops that consisted of 20 min measuring phase alternating with 2 min of flushing and 1 min of waiting phases. For the MMR estimation, in particular, we used a measuring phase of 5 min to obtain the deepest decline in oxygen uptake, which typically occurs in the first few minutes (Rosewarne et al., 2014). The oxygen concentration during the measurement phase was never below 80% to avoid any hypoxia-related effects. For calibration purposes, two-point calibration with the oxygen sensor was used to record the highest water concentration value as 100% air-saturated and the lowest

water concentration value as 0% using a solution of sodium sulphite (Na_2SO_3 , 0.159 M).

Oxygen uptake by crayfish was calculated by fitting a linear regression of the oxygen concentration decline over time. The measurement periods that yielded regressions with coefficient of determination below 0.70 were removed before data processing. The resulting slope of the linear regression was used to calculate oxygen consumption rates (MO_2 , in $\text{mg O}_2 \text{ h}^{-1}$):

$$\text{MO}_2 = -(\Delta Of - \Delta Ob) \times V$$

where ΔOf and ΔOb are the rates of oxygen consumption in $\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$ due to crayfish respiration and microbial respiration, respectively, and V is the volume of the flume respirometer (after subtracting the crayfish volume measured with a test tube). Background microbial respiration was determined before and after each experiment without crayfish for 20 min with a velocity of 6–7 cm s^{-1} to avoid stratification in the water column (Rodgers et al., 2016).

Twenty crayfish (12 females and 8 males) were used to evaluate the effects of current velocities on crayfish metabolism. We placed crayfish individually inside the flume respirometer with no flow and allowed them to habituate to experimental conditions for a period of 2 h (Rubio-Gracia et al., 2020a, b). After that, oxygen uptake measurements were performed for 20 min with step-wise increases in current velocity (i.e., 0, 10, 25, 35, and 50 cm s^{-1}). The highest rate of oxygen consumption obtained during the current velocity tests (MO_2max), which usually occurred at 35 or 50 cm s^{-1} , was used as a proxy for the maximal rate of aerobic metabolism that crayfish reach when exposed to flow.

To measure MMR, we applied the standard exhaustion protocol for a remaining group of 9 individuals (3 females and 6 males) following the method described by Rosewarne et al. (2014, 2016). Prior to measurements, each crayfish was placed in a shallow tray of water at the experimental temperature and induced to tail flip by repeatedly touching the telson. When the crayfish no longer flipped the tail, it was repeatedly turned onto its back until it did not turn right itself. At this point, the crayfish was immediately transferred to a circular tank and rotated from side to side for 5 s to rise any air bubbles trapped within the carapace. After that, the

crayfish was immediately transferred into the flume respirometer and, after a brief period of waiting phase, the oxygen consumption recordings began. MMR represents a measurement that cannot be self-regulated, and is therefore repeatable within individuals (Metcalf et al., 2016). By obtaining MMR, we could determine if the highest rate of oxygen consumption during the current velocity tests (i.e., MO_2max) approached the upper boundary of oxygen uptake that a crayfish can attain.

After the velocity tests or the MMR estimation, crayfish were retained overnight (20 h) and were allowed to rest in the flume respirometer with no disturbances. Given that we did not use an automated intermittent respirometry system, we were unable to record overnight oxygen consumptions and to identify potential effects of spontaneous activity on oxygen consumption. Crayfish are more likely to reach the minimum rates of oxygen consumption after several hours of confinement, but the duration of the resting period depends on the species (Rosewarne et al., 2016). Thus, a 20-h period was deemed sufficient to ensure that crayfish were calm (i.e., MO_2 were stabilized) (Chabot et al. 2016). During the resting period, we used a continuous current velocity of 6 cm s^{-1} , which was the minimum velocity that the equipment feature allowed. The next morning, the mean of three MO_2 measurements using intervals of 10 min were used as a proxy measure for the RMR.

Morphology

At the end of each experiment, crayfish were weighed to the nearest 0.1 mg and several morphometric variables were measured using digital calipers (0.1 mm resolution): total body length (measured from the beginning of the rostrum until the end of the telson), length and width of the right chelae, maximal cephalothorax width, abdomen width, and total uropod width.

Statistical analyses

Statistical analyses were performed in the R software environment (version 4.0.3) (R Development Core Team, 2018) and with the IBM® SPSS Statistics v.25. Prior to all analyses, all variables were \log_{10} transformed to linearize them and residual plots of

dependent variables were used to confirm the homoscedasticity and normality of residuals.

We performed one-way repeated measures ANCOVA with body mass as a covariate to compare MO_2 between current velocities (H1); the latter variable treated as a categorical factor in models. All data were pooled because there were no significant differences in MO_2 between sexes ($F_{1,16}=0.382$, $P=0.545$). The assumption of homogeneity of slopes was previously tested by analyzing the interactions between the covariate and categorical factors. If such interactions were not significant, the assumption of homogeneity of slopes was assumed and all interactions were removed from the model to improve the statistical power of the ANCOVA (García-Berthou & Moreno-Amich, 1993). Since Mauchly's test showed that the sphericity assumption of repeated-measure analyses was not satisfied, we applied the Greenhouse–Geisser correction. A Tukey's multiple comparison test (Bonferroni corrected) was then used to elucidate differences in mass-adjusted means of MO_2 between current velocities.

We performed two-way ANCOVA to compare MMR with MO_2max obtained during the current velocity tests (H2). We used two categorical factors (type of protocol and sex) in models and total body mass as a covariate. Because we used two different batches of crayfish to determine MMR and MO_2max we were unable to identify the specific amount of MMR that each individual used during the current velocity tests. However, we calculated the ratio between mass-adjusted MMR and MO_2max of the different crayfish batches to get an approximate percentage of energy used by crayfish. To do this, following previous studies (e.g., McFeeters et al., 2011; Rosewarne et al., 2014; Rubio-Gracia et al. 2020a, b), MO_2 data were corrected to a common value of body mass of 20 g (average body mass of individuals) using the equation:

$$\text{MO}_2(20\text{ g}) = \text{MO}_{2(\text{BM})} * (\text{BM}/20)^{(1-A)},$$

where $\text{MO}_{2(\text{BM})}$ is oxygen consumption of animal with body mass BM and A is the mass exponent describing the relationship between oxygen consumption and body mass. The mass exponent of the linear relationship between MO_2max and body mass was 0.54 ($\log_{10} \text{MO}_2\text{max} = -0.23 + 0.541 \log_{10} \text{body mass}$; $R^2=0.67$, $P=0.001$). As for MMR, the

linear relationship between MMR and body mass was not strong due to the reduced number of individuals used ($N=9$). Moreover, no information is available regarding the mass exponent of the linear relationship between MMR and body mass in crayfish. McFeeters et al. (2011) determined a metabolic mass-scaling exponent of 0.71 for freshwater crayfish species [*Orconectes rusticus* (Girard, 1852)] using field metabolic rate in 137 individuals of different populations. Because MMR appears to have higher scaling exponents than SMR and RMR in aquatic animals (Norin & Clark, 2016), we therefore used a common value of 0.75 for the mass exponent following previous studies (e.g., Rubio-Gracia et al., 2020a).

Sex differences in RMR and MO_2max among crayfish were assessed using one-way analysis of the variance (ANOVA) and ANCOVA (with body mass as a covariate and sex as a factor), respectively, because MO_2max scaled positively with body mass ($\log_{10} \text{MO}_2\text{max} = -0.229 + 0.542 \log_{10} \text{body mass}$; $R^2=0.454$, $F_{1,18}=14.95$, $P=0.001$), whereas RMR did not ($P=0.255$). Similarly, we performed an ANCOVA with each of the morphometric variables as a dependent variable and total body length as a covariate to assess sexual differences in morphology. Further, we performed multiple linear regressions using MO_2max as a dependent variable and morphometric variables as candidate independent variables to identify the morphological traits that have the largest influence on oxygen uptake. Subsequently, we used the significant morphometric variables, whose effects on oxygen uptake were independent of total body length, as independent variables in linear models to test whether sex-specific variation in morphology leads to differences in MO_2max between sexes (H3).

Results

During the current velocity trials, we observed that most of red swamp crayfish can tolerate water velocities up to 50 cm s^{-1} by holding a stationary position, except one small female, which was not able to maintain the upright position, and swept back against the wall of the flume respirometer. At low water velocities ($0\text{--}10 \text{ cm s}^{-1}$) individuals were able to walk, but they stopped moving at higher water velocities and maintained a stationary position by grasping both the mesh of the flume respirometer wall with the chelae

and the piece of slate at the bottom with their walking legs.

Oxygen consumption rate (MO_2) showed an increase with crayfish body size and current velocity, reaching its maximum at flow conditions ranging from 25 to 50 $cm\ s^{-1}$ (Fig. 2). Repeated measures ANCOVA showed that there was a significant interaction between current velocity and body mass (covariate) for MO_2 ($F_{2.051, 34.874} = 5.61$, $P = 0.007$), indicating that the relationship between body mass and MO_2 was not consistent among the current velocities. After adjusting for body mass, a Tukey's multiple comparison test revealed that MO_2 values measured at no flow conditions (0 $cm\ s^{-1}$) were significantly lower than MO_2 values measured at 10 $cm\ s^{-1}$, and both were significantly lower than MO_2 values measured at 25, 35, and 50 $cm\ s^{-1}$ (Fig. 3). Moreover, we found that MMR was higher than MO_{2max} based on the results of ANCOVA ($F_{1,26} = 8.10$, $P = 0.009$; Fig. 4). However, it is important to note that approximately 72% of the MMR (standardized to 20 g) was taken up by crayfish to cope with high current velocities.

We found that there were no sexual differences in both RMR ($F_{1,25} = 0.047$, $P = 0.83$) and MO_{2max} measured during the current velocity tests, after controlling for body mass ($F_{2,17} = 7.089$, $P = 0.862$). However, males weighed more ($F_{2,24} = 289.4$, $P = 0.008$), had longer ($F_{2,20} = 27.95$, $P = 0.002$) and wider ($F_{2,20} = 58.49$, $P = 0.0001$) chelae, and wider cephalothorax ($F_{2,24} = 397.8$, $P = 0.028$) than females

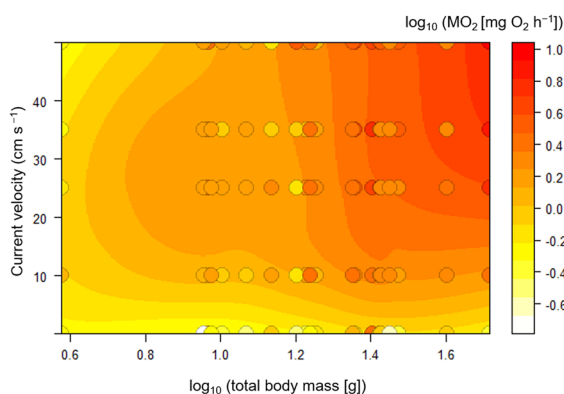


Fig. 2 Surface plots relating oxygen consumption rate (MO_2) with body mass and current velocity (0, 10, 25, 35, and 50 $cm\ s^{-1}$) for the red swamp crayfish (*Procambarus clarkii*). Each individual contributed to one data point for each current velocity. Note \log_{10} -transformations for MO_2 and body mass variables

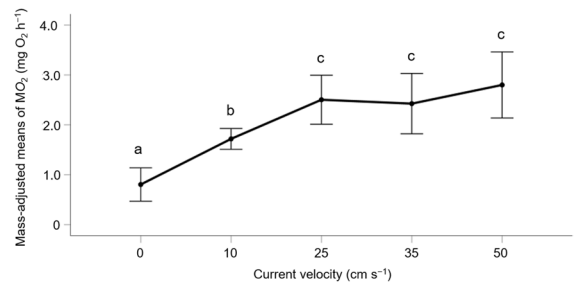


Fig. 3 Covariate-adjusted means ($\pm 95\%$ CI) of oxygen uptake rates (MO_2 , $mg\ O_2\ h^{-1}$) of red swamp crayfish at different current velocities ($cm\ s^{-1}$). Different lower case letters denote significant differences in the mean values ($P < 0.05$)

(Fig. 5A–D). By contrast, both sexes showed similar size of abdomen width ($F_{2,24} = 470.8$, $P = 0.076$) and total uropod width ($F_{2,24} = 79.74$, $P = 0.556$). Moreover, MO_{2max} scaled positively with several morphometric variables (\log_{10} - \log_{10} relationships), such as total body length ($R^2 = 0.406$, $F_{1,18} = 12.29$, $P = 0.003$), total uropod width ($R^2 = 0.208$, $F_{1,18} = 4.74$, $P = 0.043$), abdomen width ($R^2 = 0.422$, $F_{1,18} = 13.13$, $P = 0.002$), and cephalothorax width ($R^2 = 0.487$, $F_{1,18} = 17.11$, $P = 0.0001$; Fig. 6). However, our multiple regression models showed that, with the exemption of cephalothorax width (i.e., cephalothorax width was significant in models but not total body length), the rest of morphological variables were size-dependent. Then, we performed ANCOVA to assess sexual differences in MO_{2max} when accounting for cephalothorax width. Our results showed that the sexual dimorphism in cephalothorax width did not lead to sexual differences in MO_{2max} ($F_{2,17} = 8.211$, $P = 0.717$).

Discussion

As far as we know, this is the first study to evaluate the effects of current velocity on oxygen uptake in the red swamp crayfish. This information can provide a mechanistic understanding of the physiological limitations of this invasive crayfish in relation to water flow. Our results suggest that a direct exposure of water velocity of at least 25 $cm\ s^{-1}$ could compromise the success of crayfish populations through excessive flow-driven energetic demands. This would partially explain the failed invasions of the red swamp crayfish

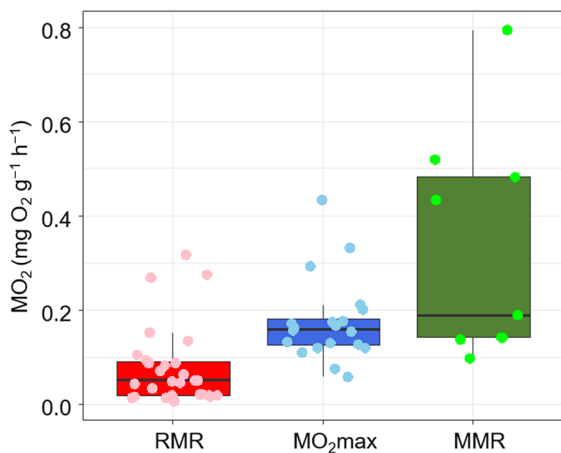


Fig. 4 Box plot showing mass-specific means and confidence intervals ($\pm 95\%$) of routine metabolic rate (RMR), maximum metabolic rate (MMR), and the highest rate of oxygen consumption obtained during the current velocity tests (MO_2max). Boxplots represent the minimum, first quartile (Q1), median, third quartile (Q3) and maximum, and dots represent individual data points (in $mg\ O_2\ h^{-1}\ g^{-1}$)

in fast-flowing waters in its introduced range (e.g., Cruz & Rebelo, 2007; Maceda-Veiga et al., 2013), but also the preference of this species for lentic waters in streams and for stagnant waters in lakes and ponds (van Kuijk et al. 2021). Preventing or reducing the dispersion of invasive crayfishes is a key management concern (Freeman et al., 2010; Vedia & Miranda, 2013; Crook et al., 2015). Appropriate flow management actions (e.g., regular flow releases from weirs; Chester et al., 2014) and the restoration of natural flow regimes, e.g., by removing physical barriers that lower stream discharge downstream, can be thus of critical importance for controlling the spread of invasive crayfish populations. According to our findings, the effectiveness of these measures may be dependent on maintaining water velocities in streams higher than $25\ cm\ s^{-1}$, considering that current velocity can substantially vary at the microhabitat scale due to the presence of stones and aquatic vegetation.

In agreement with our hypothesis (H1), oxygen uptake by crayfish increased with increasing current velocities, as drag increases exponentially with water velocity in crayfish (Maude & Williams, 1983). However, the increase in oxygen uptake was also related to the size of individuals (thus supporting H3). That is, larger crayfish showed higher capacity of oxygen uptake than smaller crayfish, especially at

high current velocities. Previous studies showed that elevated water velocity appeared to differentially affect large over small crayfish, as small crayfish can more easily crawl into stone interstices to avoid high hydraulic stress (Maude & Williams, 1983; Clark et al., 2008). Moreover, Clark et al. (2008) found that small Allegheny crayfish [*Orconectes obscurus* (Hagen, 1870)] occupied large-substratum, fast water-velocity riffles, whereas larger crayfish occupied small-substratum, lower-velocity pools. Our results are in line with these size-dependent effects and suggest that larger red swamp crayfish could be more affected by high current velocities due to the increased energy expenditure to overcome drag (Maude & William, 1983). This hydrodynamical disadvantage in high-drag morphs agrees with the diversity and plasticity of morphological phenotypes in the field, with crayfish from high-velocity streams having a more fusiform body shape than crayfish from low-velocity streams and lakes (Perry et al., 2013).

We also found that RMR did not scale positively with body mass under resting conditions. Conversely, Toro-Chacon et al. (2021) found a significant relationship between body mass (range body mass: 0.24–42.93 g) and RMR in another burrowing freshwater crayfish [*Parastacus pugnax* (Poeppig, 1835)]. These contradictory results may be related to the smaller range of body mass used in our study, but also to the use of different respirometry methods and systems (static respirometry chamber vs flume respirometer, e.g., see Rummer et al., 2016). Moreover, we acknowledge that measuring MO_2 overnight produces more accurate estimates of RMR because it is possible to exclude outlier data points and account for spontaneous activity (Chabot et al., 2016).

We hypothesized that the red swamp crayfish would reach the upper level of oxygen uptake when exposed to high current velocities (H2). However, contrary to expectations, our results showed that MO_2max was lower than MMR, which represents the highest aerobic metabolic rate attainable by an organism (Rosewarne et al., 2016; Norin & Clark, 2016). Even so, we found evidence that crayfish might have operated close to their maximum aerobic capacity (i.e., oxygen uptake represented more than 70% of the MMR). This finding indicates that most of their aerobic energy budget was required to cope with the extreme oxygen demand associated with high water flows. Thus, only a remaining 30% of the energy

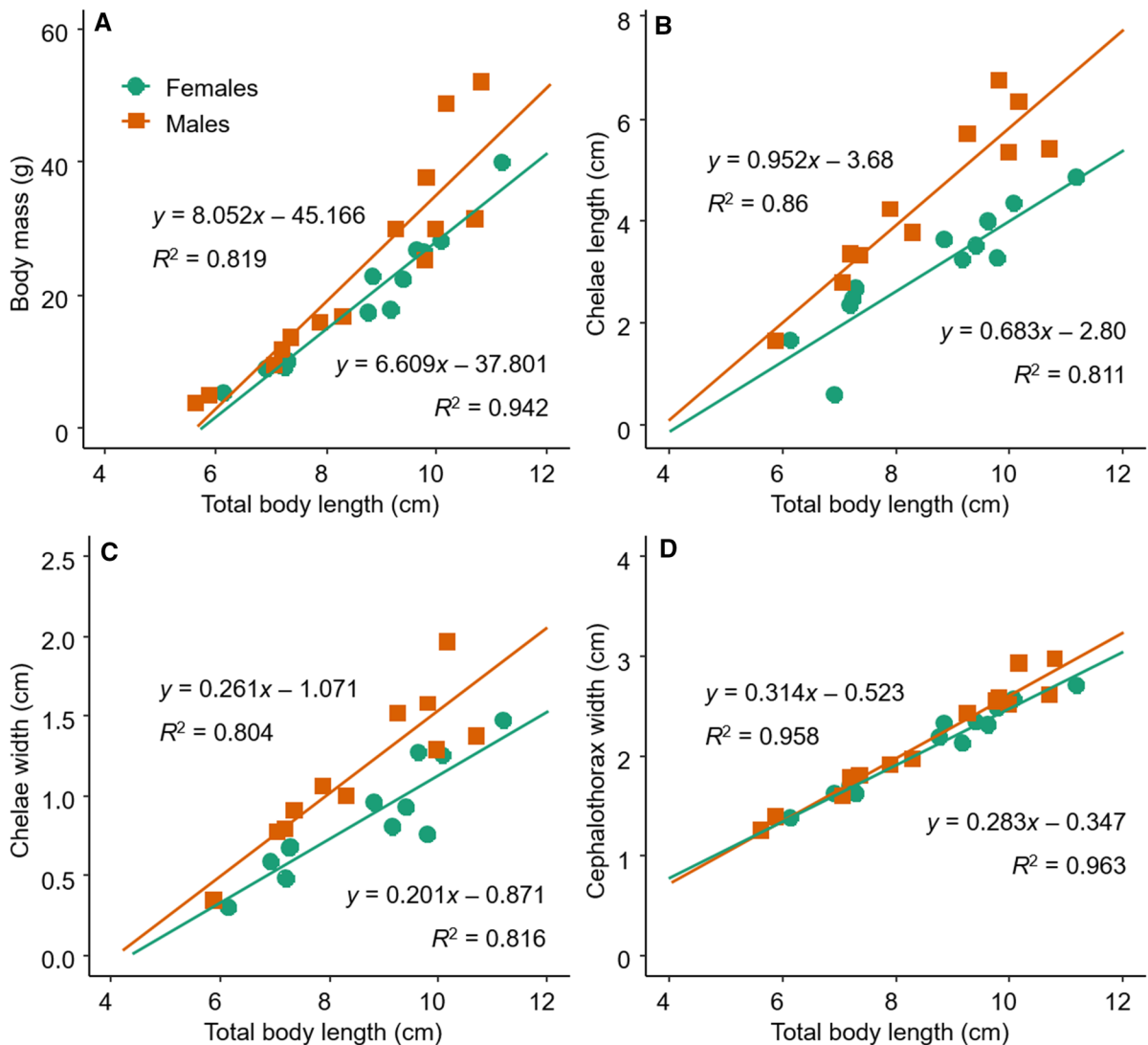


Fig. 5 Body mass (A), right chelae length (B) and width (C) and cephalothorax width (D) as a function of total body length for males and females of the red swamp crayfish (*Procambarus clarkii*)

budget was available for crayfish to perform other activities such as activity, digestion, and reproduction (Clark et al. 2013). We therefore suggest that the crayfish exposure to prolonged high flow conditions would potentially affect the fitness of their populations. In fact, vital activities such as foraging, spawning, mating, and growth are closely linked to flow regimes for several crayfish species (Lowery, 1988; Mead, 2008; Barnett et al., 2017). In this sense, in a similar study using a laboratory-recirculating flume, Perry & Jones (2018) demonstrated a 30–55% loss of performance in rusty crayfish (*Orconectes rusticus*)

attempting to reach food in high-velocity flows (66 cm s^{-1}), regardless of body size. Nonetheless, it is important to note that laboratory conditions may not reflect real habitat complexity in the wild. Because this crayfish species burrows through mud (Souty-Grosset et al., 2016) and has the ability to move overland distances up to 1 km (Kerby et al., 2005; Cruz & Rebelo, 2007; Banha & Anastácio, 2014), their metabolic constraints could not necessarily affect their dispersal capacity along the stream. In addition, many invasive crayfish species have wide physiological tolerances, flexible behaviors, high dispersal abilities,

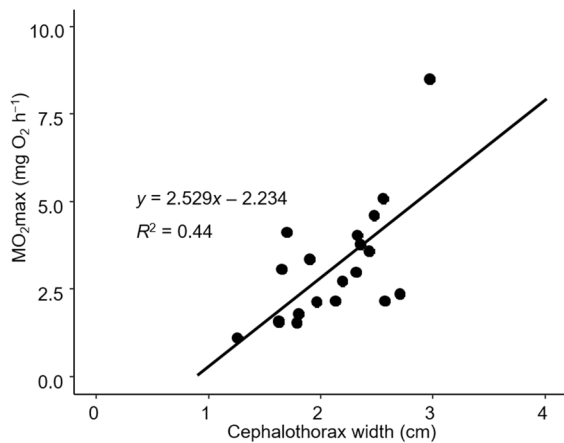


Fig. 6 Linear relationship between cephalothorax width and the highest rate of oxygen consumption obtained during the velocity tests ($MO_2\max$)

and high phenotypic plasticity (e.g., morphology) allowing them to establish in new habitats (Perry et al., 2013; Crook et al., 2015; Zeng et al., 2015). Therefore, our results should be interpreted with caution and future studies integrating physiological and functional traits, population dynamics, and stream flow conditions are needed to understand the consequences of flow regime variability on invasive crayfish populations.

Our results showed that males have larger and broader right chelae and wider cephalothorax than females but, contrary to expectations (H3), these morphological differences did not lead to sexual differences in oxygen consumption rates. Moreover, among all morphological variables, only cephalothorax width influenced oxygen uptake by crayfish (i.e., $MO_2\max$ increased with cephalothorax width), after accounting for body size. The effects of crayfish body parts on hydrodynamics are not clear and it seems to vary depending on the species. Perry et al. (2013) suggested that the chelae size in *O. rusticus* can play a key role in deflecting water over the bodies, thus decreasing drag and hence metabolic costs. However, other studies found that the larger size of the males' chelae in other crayfish species [e.g., *Uca pugilator* (Bosc, 1801)] can impose significant metabolic costs (i.e., increased blood lactate levels) due to greater drag (Tullis & Straube, 2017) and reduce tail-flip escape response (Robinson & Gifford, 2019; Hundayi et al., 2020).

In our study, the apparent similar levels of oxygen uptake (after adjusting for body size effects) between current velocities (25–50 cm s^{-1}) and the small effects of morphological traits on $MO_2\max$ could be related to the stationary position that crayfish maintained into the flume respirometer. In fact, the impact of exaggerated structures of crayfish such as a large chelae on energy expenditure may depend on exercise intensity and strenuous locomotion, as previous studies noted (e.g., Tullis & Straube, 2017).

In short, this study would make an important contribution toward better understanding the physiological responses of the invasive red swamp crayfish associated with varying flow conditions, and the extent and underlying causes of variation. Our results showed that high current velocities ($\geq 25 \text{ cm s}^{-1}$) could generate excessive energetic costs to the red swamp crayfish, thus compromising the animal's capacity to simultaneously supply oxygen to energy-demanding fitness traits such as activity, reproduction, and growth. These metabolic constraints could partly explain the failed invasions of this invasive crayfish species in fast-flowing waters. Moreover, sexually dimorphic traits in the red swamp crayfish did not cause significant changes in oxygen uptake among individuals. In contrast, crayfish body size was the most influential predictor of flow-driven metabolic costs, as larger crayfish expended more energy to overcome drag and maintain station at higher current velocities. These findings may be useful for water management decision processes to control and prevent the spread of invasive crayfish in its introduced range.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This research was approved by the Ethics Committee of the University of Girona.

Consent to participate All authors consent to participate in this research.

Consent for publication All authors consent to publish this research.

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