Critical swimming speed at different temperatures for small-bodied freshwater native riverine fish species

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Abstract

This study evaluated the effect of fish total length ($L_T$) and three water temperatures (10, 15 and 20 °C) on the critical swimming speed ($U_{crit}$) of the species *Percilia irwini* (2.9 – 6.3 cm $L_T$), *Cheirodon galusdae* (3.4 – 5.5 cm $L_T$), and *Trichomycterus areolatus* (4.0 – 6.3 cm $L_T$). An $U_{crit}$ estimation model was constructed for each species as a function of temperature and size. The results showed mean $U_{crit}$ for *P. irwini* of 44.56, 53.83 and 63.2 cm s$^{-1}$ at 10, 15 and 20 °C, respectively: 55.34, 61.74 and 70.05 cm s$^{-1}$ for *C. galusdae* and 56.18, 63.01 and 71.09 cm s$^{-1}$ for *T. areolatus*. Critical velocity depended on the interaction between species, body length and water. The swimming performance increased significantly with rising temperature in all three species. The velocity also increased with greater LT. After controlling for LT, velocity also increased with higher temperature in the three species. This research is relevant to small fish species that require conservation measures.

Introduction

Possible extinction of fish in rivers depends on their life-history and their interaction with the ecosystem's environmental conditions (Bergerot et al. 2015). Swimming performance is an important factor for survival of fish (Tudorache et al. 2008), distribution, migration, predator-prey interactions and reproduction (Wolter and Arlinghaus 2003). The movement within habitats allow fish to optimize access to resources, refuges, gene flow, reproduction and colonization of new territories (Albanese et al. 2004).

Swimming speed and hydrodynamic resistance in fish varies according to the type of species, morphology, size, water temperature, oxygen levels, water quality and other variables (Hammer 1995; Plaut 2000; Tudorache et al. 2008; Zeng et al. 2009). However, temperature significantly influences the physiological functions and behavior of aquatic animals (Lee et al. 2003; MacNutt et al. 2004). Temperature fluctuations have a profound effect on swimming capacity (Beamish 1978; Lee et al. 2003; Zeng et al. 2009; Yan et al. 2012), influencing cardiovascular capacity and the respiratory system that sustains aerobic metabolism (Farrell et al. 1996). The body length of fish influences the strength and resistance to movement (Boily and Magnan, 2002).

Critical swimming speed ($U_{crit}$) is a special category of prolonged swimming used to estimate maximum sustained speed (Brett 1964). It is defined as the highest swimming speed that a fish can maintain for a period equal in magnitude to the time interval used in the test (Peake et al. 1997). It is measured by confining fish in a respirometer, which does not necessarily mimic swimming in the wild and should therefore be used cautiously (Cano-Barbacil et al. 2020). The relationship between temperature and $U_{crit}$ speed follows a normal distribution (Claireaux et al. 2006, Lee et al. 2003; MacNutt et al. 2004).

Most studies on swimming capacity have been performed in laboratories with swim tunnel respirometers (Hammer 1995; Katopodis 2005), and in raceways (Colavecchia et al. 1998) with fixed water temperature. Therefore, it is necessary to evaluate swimming velocity considering the different factors that may affect it, such as water temperature and total length (Silva et al. 2018).
This study evaluates the swimming capacity of three native small-bodied freshwater fish species from Chile ($L_T<12$ cm); *Percilia irwini* (Eigenmann, 1927), *Cheirodon galusdae* (Eigenmann, 1928), and *Trichomycterus areolatus* (Valenciennes 1840). They are all endemic species that inhabit high flow rivers in central Chile whose substrata have boulders mixed with sand (Arratia et al. 1983; Habit and Belk, 2007; García et al. 2012). In Chile these species are classified as vulnerable (*C. galusdae* and *T. areolatus*), and endangered (*P. irwini*) (Ministerio de Medio Ambiente 2023). In addition, most studies have been done on salmonids such as rainbow trout *Oncorhynchus mykiss* (Walbaum 1972), brown trout *Salmo trutta* (Linnaeus 1758), sockeye salmon *O. nerka* (Walbaum 1972) and coho salmon *O. kisutch* (Walbaum 1972) (Birnie-Gauvien et al. 2019; Gregory and Wood 1998; Lee et al. 2003; Ojanguren and Braña 2005), which are all introduced species in Chilean ecosystems (Arismendi et al. 2014). However, swimming studies in native Chilean fish are scarce.

The objective of this study was to provide the swimming capacity of this native species by measuring critical swimming speed. The effect of species, fish lengths and water temperatures, on swimming performance was investigated.

**Results**

**Critical swimming speed**

Critical swimming speeds and relative critical swimming speed at different temperatures for three species were ranging from 22.19 to 98.91 cm s$^{-1}$ for *P. irwini*, from 45.68 to 92.73 cm s$^{-1}$ for *C. galusdae*, and from 41.75 to 93.57 cm s$^{-1}$ for *T. areolatus* (Fig. 1 (a)). The relative critical velocities (Fig.1 (b)) increased significantly with rising temperature in all three species.

We found that critical velocity depended on the interaction between species, body length and temperature (Table 1). These results indicate that the effect of the temperature differed through body length. There was greater dispersion in the critical velocity in *P. irwini* than in the other two species.

**Table 1.** PERMANOVA of velocity $U_{crit}$ (cm s$^{-1}$) (species (S):fixed, water temperature(T): fixed, and length ($L_T$): random). Probabilities associated at each ratios *F-ratio* were obtained with 9999 permutations on residual under a reduced model.
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F-ratio</th>
<th>p-value</th>
<th>Öc.v</th>
<th>%c.v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specie (Sp)</td>
<td>2</td>
<td>1675.7</td>
<td>15.297</td>
<td>&lt;0.001</td>
<td>4.99</td>
<td>12</td>
</tr>
<tr>
<td>Water Temperature (T)</td>
<td>2</td>
<td>5120.1</td>
<td>59.582</td>
<td>&lt;0.001</td>
<td>7.44</td>
<td>17</td>
</tr>
<tr>
<td>Length (L_T)</td>
<td>23</td>
<td>2285.2</td>
<td>296.09</td>
<td>&lt;0.001</td>
<td>12.9</td>
<td>30</td>
</tr>
<tr>
<td>SpxT</td>
<td>4</td>
<td>195.2</td>
<td>2.867</td>
<td>&lt;0.05</td>
<td>2.46</td>
<td>6</td>
</tr>
<tr>
<td>SpxL_T</td>
<td>12</td>
<td>109.5</td>
<td>14.194</td>
<td>&lt;0.001</td>
<td>3.36</td>
<td>8</td>
</tr>
<tr>
<td>TxL_T</td>
<td>46</td>
<td>101.9</td>
<td>13.209</td>
<td>&lt;0.001</td>
<td>4.54</td>
<td>11</td>
</tr>
<tr>
<td>SpxTxL_T</td>
<td>24</td>
<td>68.1</td>
<td>8.82</td>
<td>&lt;0.001</td>
<td>4.49</td>
<td>10</td>
</tr>
<tr>
<td>Residuals</td>
<td>341</td>
<td>7.7</td>
<td>2.78</td>
<td></td>
<td>2.78</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold values represent statistically significant values with alpha=0.05

In all three species, the speeds depend significantly on the interaction between the temperature and the length of the fish (Table 2). Pair-wise comparisons between test temperatures showed significant differences across all species and temperature levels (Table 3). Patterns of $U_{crit}$ (cm s$^{-1}$) were statistically different among length of the fishes in *P. irwini* (PERMDISP, $F_{14,120}$=3.43; $p<0.001$), *C. galusade* (PERMDISP, $F_{11,96}$=8.83; $p<0.001$) and *T. areolatus* (PERMDISP, $F_{10,88}$=3.9; $p<0.001$).

**Table 2.** PERMANOVA based on Euclidean dissimilarity measure for velocity $U_{crit}$ (cm s$^{-1}$) by species (water temperature (T): fixed, and length (L_T): random). Probabilities associated at each ratios *F-ratio* were obtained with 9999 permutations on residual under a reduced model.
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F-ratio</th>
<th>p-value</th>
<th>c.v</th>
<th>%c.v</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percilia irwini</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Temperature (T)</td>
<td>2</td>
<td>3909</td>
<td>42.136</td>
<td>&lt;0.001</td>
<td>9.21</td>
<td>27</td>
</tr>
<tr>
<td>Length (L&lt;sub&gt;T&lt;/sub&gt;)</td>
<td>14</td>
<td>2370.6</td>
<td>215.11</td>
<td>&lt;0.001</td>
<td>16.19</td>
<td>48</td>
</tr>
<tr>
<td>Tx L&lt;sub&gt;T&lt;/sub&gt;</td>
<td>28</td>
<td>92.77</td>
<td>8.418</td>
<td>&lt;0.001</td>
<td>5.22</td>
<td>15</td>
</tr>
<tr>
<td>Residuals</td>
<td>90</td>
<td>11.02</td>
<td>3.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cheirodon galusdae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Temperature (T)</td>
<td>2</td>
<td>1957.2</td>
<td>15.28</td>
<td>&lt;0.001</td>
<td>7.12</td>
<td>29</td>
</tr>
<tr>
<td>Length (L&lt;sub&gt;T&lt;/sub&gt;)</td>
<td>11</td>
<td>734.7</td>
<td>216.13</td>
<td>&lt;0.001</td>
<td>9.01</td>
<td>37</td>
</tr>
<tr>
<td>Tx L&lt;sub&gt;T&lt;/sub&gt;</td>
<td>22</td>
<td>128.13</td>
<td>37.7</td>
<td>&lt;0.001</td>
<td>6.44</td>
<td>26</td>
</tr>
<tr>
<td>Residuals</td>
<td>72</td>
<td>3.39</td>
<td>1.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trichomycterus areolatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Temperature (T)</td>
<td>2</td>
<td>1838.8</td>
<td>40.55</td>
<td>&lt;0.001</td>
<td>7.37</td>
<td>29</td>
</tr>
<tr>
<td>Length (L&lt;sub&gt;T&lt;/sub&gt;)</td>
<td>10</td>
<td>1260.4</td>
<td>159.02</td>
<td>&lt;0.001</td>
<td>11.80</td>
<td>46</td>
</tr>
<tr>
<td>Tx L&lt;sub&gt;T&lt;/sub&gt;</td>
<td>20</td>
<td>45.35</td>
<td>5.722</td>
<td>&lt;0.001</td>
<td>3.53</td>
<td>14</td>
</tr>
<tr>
<td>Residuals</td>
<td>66</td>
<td>7.93</td>
<td>2.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold values represent statistically significant values with alpha=0.05

**Table 3.** PERMANOVA pair-wise comparisons among water temperature for each species on the basis of the Euclidean dissimilarities on velocity $U_{\text{crit}}$ (cm s<sup>-1</sup>).

<table>
<thead>
<tr>
<th>Specie</th>
<th>Water Temperature (°C)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percilia irwini</strong></td>
<td>10 – 15</td>
<td>8.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>10 – 20</td>
<td>2.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15 - 20</td>
<td>3.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Cheirodon galusdae</strong></td>
<td>10 – 15</td>
<td>4.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10 – 20</td>
<td>4.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15 - 20</td>
<td>2.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Trichomycterus areolatus</strong></td>
<td>10 – 15</td>
<td>7.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>10 – 20</td>
<td>7.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15 - 20</td>
<td>4.40</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Bold values represent statistically significant values with alpha=0.05

$U_{\text{crit}}$ models
The regressions between $U_{\text{crit}}$ and fish length present linear fit in *P. irwini* and *C. galusdae* and a power function in *T. areolatus* is shown in Table 4.

**Table 4.** Adjusted equations for critical velocity $U_{\text{crit}}$ (cm s$^{-1}$) per species based on total length $L_T$ (cm).

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>Forms of regression</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Percilia irwini</em></td>
<td>10</td>
<td>Linear</td>
<td>-12.59</td>
<td>12.95</td>
<td>0.87</td>
<td>307.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Linear</td>
<td>-6.8</td>
<td>13.73</td>
<td>0.82</td>
<td>203.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>-12.57</td>
<td>17.2</td>
<td>0.89</td>
<td>360.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Cheirodon galusdae</em></td>
<td>10</td>
<td>Linear</td>
<td>9.36</td>
<td>10.41</td>
<td>0.57</td>
<td>44.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Linear</td>
<td>1.44</td>
<td>13.7</td>
<td>0.71</td>
<td>88.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>-10.1</td>
<td>18.14</td>
<td>0.72</td>
<td>93.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Trichomycterus areolatus</em></td>
<td>10</td>
<td>Power function</td>
<td>2.3</td>
<td>1.05</td>
<td>0.76</td>
<td>103.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Power function</td>
<td>2.5</td>
<td>1.03</td>
<td>0.74</td>
<td>95.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>1.93</td>
<td>1.41</td>
<td>0.92</td>
<td>375.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Linear regression $U_{\text{crit}} = a + b \times L_T$ and power function $\ln(U_{\text{crit}}) = a + b \times \ln(L_T)$. Bold values represent statistically significant values with alpha=0.05.

The relationship between the natural logarithm of critical velocity (ln $U_{\text{crit}}$) and water temperature and fish length showed a significant fit in the three species (Table 5). The coefficients for temperature and body length were positive, showing their direct proportionality to the three species critical velocity. It can be observed that as temperature increases, velocity increases in *P. irwini* (parameter $b$ in Table 5), while increases in velocity for *C. galusdae* and *T. areolatus* were similar. The increase in velocity as body length increases (parameter $c$ in Table 5) was less pronounced in *C. galusdae*, while *P. irwini* and *T. areolatus* were similar.

**Table 5.** Adjusted equations for critical velocity $U_{\text{crit}}$ (cm s$^{-1}$) per species based on total length $L_T$ (cm) and water temperature $W_T$ (°C): $\ln(U_{\text{crit}}$ (cm s$^{-1}$)) = $a + b \times W_T$ (°C) + $c \ln(L_T$ (cm)).
<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$R^2$</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Percilia irwini</em></td>
<td>135</td>
<td>1.672</td>
<td>0.0355</td>
<td>1.184</td>
<td>0.88</td>
<td>489.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Cheirodon galusdae</em></td>
<td>108</td>
<td>2.362</td>
<td>0.0229</td>
<td>0.955</td>
<td>0.74</td>
<td>149.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Trichomycterus areolatus</em></td>
<td>99</td>
<td>1.881</td>
<td>0.0230</td>
<td>1.164</td>
<td>0.88</td>
<td>273.26</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Bold values represent statistically significant values with alpha=0.05

**Discussion**

This is the first study that considers temperature as a factor affecting critical velocity in three native Chilean species. The results show the importance of considering water temperature as a predictor of critical speed, being necessary in the future to evaluate other factors such as the body shape of the fish (Cano-Barbacil et al. 2020). The differences in swimming performance at different temperatures provide significant information to understand fish distribution (Buisson et al. 2008). Knowing swimming velocity behavior at different temperatures and body lengths of small native fish allows for better conservation decisions, for example in the design of fishways for anthropic interventions in rivers (Rodgers et al. 2014; Hoagstrom 2015; Laborde et al. 2016).

The tests performed on the three native fish at three water temperatures showed an increase in $U_{crit}$ as the temperature increased and were different (Table 3), similar to what has been found for other fish species including guppies *Poecilia reticulata* (Peters, 1859) (Kent and Ojanguren, 2015), juvenile Australian bass *Percalates novemaculeata* (Steindachner 1866) and empire gudgeon *Hypseleotris compressa* (Krefft, 1864) (Rodgers et al. 2014), juvenile Chinese aturgeon *Acipenser sinensis* (Gray 1835) (He et al. 2013), giant danios *Devario aequipinnatus* (McClelland, 1839) (Bartolini et al. 2015), European bass *Dicentrarchus labrax* (Linnaeus, 1758) (Claireaux et al. 2006) and catfish *Silurus meridionalis* (Chen, 1977) (Zeng et al. 2009). In the present study, the increase in the average relative speed with increasing water temperature was 17.89 in *P. irwini*, 13.78 in *C. galusdae* and 12.83 in *T. areolatus*, values higher than those found in small-bodied fishes by Rodgers et al. (2014).

Considering water temperature and fish body length together, the $U_{crit}$ value was greater in *C. galusdae* than in *P. irwini*, which in turn was greater than the value for *T. areolatus* (Table 1). These three species are allopatric in rivers with current velocities between 0.2 and 300 cm s$^{-1}$. They use different habitats within the rivers, the catfish *T. areolatus*, for example, uses the bottom more frequently (Arratia, 1983). Differences in swimming speed have been reported between species with anguilliform swimming such as *T. areolatus* and subcarangiform and carangiform species such as *P. irwini* and *C. galusdae* (Katopodis and Gervais 2016).

The mean $U_{crit}$ values found in this analysis are similar to those found in other studies of freshwater fish with similar body length at a water test temperature between 15 and 20°C (Egger et al. 2021; Zhao et al. 2018).
The results for *C. galusdae* at 15 and 20°C were like those found by Laborde et al. (2016) using a fixed water test temperature of 17°C.

The $U_{\text{crit}}$ model estimated including temperature (Table 5) shows a significant relationship similar to those found by Cano-Barbacil et al. (2020). When the effect of $L_T$ is isolated (Fig. 1 (b)), speed also increases with temperature. As has been previously reported in several studies of freshwater fish (Cai et al. 2020; Starrs et al. 2011; Zupa et al. 2015), at a given temperature, critical velocity increased as the studied species $L_T$ increased. There was a linear relationship between critical velocity and $L_T$, similar to what was reported by Hou et al. (2018). Thus, fish $L_T$ is relevant when estimating critical velocity (Table 5 and 6), increasing at larger sizes as has been observed in many studies (Mateus et al. 2008; Mu et al. 2019; Rodgers et al. 2014; Zupa et al. 2015; Cano-Barbacil et al. 2020).

We found a positive relationship between $U_{\text{crit}}$ and $L_T$ in the three studied species (Table 4), which could be related to increased muscle mass and metabolism during the swim (Beamish 1978; Peake 2008). The $U_{\text{crit}}$ model estimated by temperature for the three species (Table 4 and 5) shows a positive linear increase with $L_T$ for *P. irwini* and *C. galusdae*, with a best fit than other studies for carp fishes (Tan et al. 2021) and for European sea bass (Zupa et al. 2015). For *T. areolatus* a power function was the one with the best fit as found by Cano-Barbacil et al. (2020) for most Iberian dish species.

However, this estimated function is only valid for the tested range of temperatures; at higher temperatures we would expect the swimming velocity to decrease (Pang et al. 2011). The rivers inhabited by the studied species may reach temperatures between 9 and 25°C in pools and shallow areas with low current velocity (Dirección General de Aguas 2018); a wider range of temperatures might result in a bell-shaped curve, and should be further investigated.

Our results are conservative, since swimming capacity may be underestimated in the laboratory (Peake 2004; Egger et al. 2021). Fish swimming behavior may change in the wild, depending on the time of year, their reproductive status and the medium’s hydrodynamic conditions. Therefore, the velocity values must be validated in natural conditions. The results of this study are relevant, given the little research in small-bodied fish in Chile with conservation problem, and other countries with similar species (Habit et al. 2018; Marsden and Stuart 2019; Pompeau et al. 2012; Wolter and Schomaker 2019).

**Methods**

**Fish sampling and acclimatization**

Individuals of *P. irwini* and *C. galusdae* were collected from the Andalién River (36°49'4.13"S, 72°51'11.89"W) and *T. areolatus* from the Itata River (36°41'8.22"S, 72°26'45.31"O), between November 2017 (spring) and January 2018 (summer). Both rivers are located in the Biobío Region and Ñuble Region, in Chile (see Fig. 2). Fish were caught using a Halltech backpack electrofishing (Halltech Environmental Inc., Guelph, ON, Canada) and transported into bags and then sent to the Ecohydraulics
Laboratory at the Universidad Católica de la Santísima Concepción. The minimum sample size defined for each species was 11 fish of different total body length and three specimens of similar size, with a total of 33 fish per species. The sample number was increased when at least three specimens of a different total body length were captured. Total body length (cm) and the weight of each fish was measured by vernier caliper and electronic scale respectively (Table 6).

All fish were housed in an aquarium using the methodology of Sobones et al. (2012). Fish were separated by species, and acclimated to the lowest test temperature gradually (10°C). Each fish was placed in a closed plastic bag with 300 ml of river water, and placed in a tank with water at 10°C for 30 minutes. Then, the bags were opened and mixed with 300 ml of water from the reservoir for 15 minutes, and placed in an aquarium with water from the laboratory at 10°C.

They were fed ad libitum with Enchitrea sp. daily, which was interrupted 48 hours before each experiment. Tests were performed at 10, 15 and 20°C, based on the temperature ranges observed in the two rivers (Monsalve et al. 2012; Pedreros et al. 2013). Fish were acclimated to the test temperature for at least 15 days before the trial. All fish were returned in good health to their habitats after the experiments.

**Table 6.** Length (cm) and weight (g) of fish species in trials. N=number of fish; s.e.=standard error.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Total length (cm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>mean ± s.e.</td>
</tr>
<tr>
<td>Percilia irwini</td>
<td>45</td>
<td>2.9 – 6.3</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>Cheirodon galusdae</td>
<td>36</td>
<td>3.4–5.5</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Trichomycterus areolatus</td>
<td>33</td>
<td>4.0-6.3</td>
<td>5.2 ± 0.8</td>
</tr>
</tbody>
</table>

**Swimming trials**

Swimming trials were carried out in a Steffensen type respirometer (20 L, 4 L swim chamber: 40 cm x 10 cm x 40 cm), submerged in an 80 L tank to maintain a constant temperature. Fish were selected randomly, and once the swimming trial was finished they were separated for 24 hours to begin the acclimatization process to the next trial temperature.

Total swimming time until fatigue and water velocity at fatigue were recorded to calculate critical swimming speed $U_{crit}$ using Brett’s (1964) equation:

$$U_{crit} = U_f + \frac{T_f}{T_i} U_t \quad (1)$$
where $U_f$ is penultimate velocity (cm s$^{-1}$), $U_i$ is the water velocity increment (0.5 of the $L_T$ in cm s$^{-1}$), $T_f$ is the time swum in the final increment and $T_i$ is the time interval (300 s). This provide a measurement of the maximum velocity at which a fish can sustainably swim without fatiguing (Hammer 1995; Peake 2004).

The obstruction of the water flow by fish was negligible, since the cross-sectional area of the fish was less than 10% of the cross-section area of the test chamber (Webb 1971). The critical velocity was evaluated for each fish, for each of the three temperatures, obtaining a total of 342 velocity records for the three species. Fish swimming e was compared between standardized total length, $U_{crit}$ (cm s$^{-1}$) divided by $L_T$ (cm) and denoted as relative critical swimming speed ($R \frac{U_{crit}}{L_T}$)).

**Statistical Analysis**

To compare critical velocity between species and for each species, it was used a permutational analysis of variance with 9999 permutations (PERMANOVA), with the fixed factors species and temperature and the random factor body length. A pair-wise test were also performed when significant differences were observed between fix factors. Differences in dispersion between fish body length were analyzed using permutational analyses of multivariate dispersion (PERMDISP). This analysis was performed for each species. The multivariate analyses were carried out using Euclidean distance on critical velocity data. The PERMANOVA analyses were performed with PRIMER v7 (Anderson et al. 2008). The significance level to reject the null hypotheses was set at 0.05.

To understand the relationship between $U_{crit}$ (cm s$^{-1}$) of each species with the total length ($L_T$) by water temperature, different forms of regression were estimated, selecting the form with the best regression fit $R^2$.

The relationship between the $U_{crit}$ (cm s$^{-1}$) of each species to total length ($L_T$) and water temperature (WT($°C$)) was estimated using a linear function based on Williams and Brett (1987) *sensu* Hammer (1995):

$$\ln U_{crit} = a + b \text{WT}(°C) + c \ln (L_T(\text{cm}))$$

(2)

where $a$, $b$, and $c$ are parameters estimated from the multivariate regression.

**Declarations**

**Ethics approval**

The experimental protocols were approved and financed by the Dirección de Investigación de la Universidad Católica de la Santísima Concepción through the DIN-UCSC 10/2014 project. The care and use of experimental animals complied with Subsecretaría de Pesca y Acuicultura of the Ministerio de
Economía, Fomento y Turismo of Chile, animal welfare laws, guidelines and policies as approved by Res. Ex N° 3542, 2014. All methods were executed in accordance with ARRIVE guidelines.

Data Availability

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

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Author contributions

C.S and C.D design the research, F.S. run the experiments, collected the data, performed the data analyses, C.S and C.D wrote the paper and data analysis.

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Competing interests

The authors declare no competing interests.

Additional Information

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References


Figures

Figure 1

Maximum, minimum, mean (x), median (horizontal bar) quartile and outlier values of critical velocity $U_{crit}$ expressed in cm s$^{-1}$ (a) and relative critical swimming speed ($RU_{crit}$) expressed in $L_T$ s$^{-1}$ (b) for the three
native species tested at three water temperatures (10, 15 and 20°C).

Figure 2

Map of the study sites in the Andalién and Itata rivers, Chile.