The freshwater shrimp *Atyaephyra desmarestii* (Millet, 1831) as a bioindicator of hypoxic event effects on temperate freshwater systems

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1 Abstract

2 In order to assess the suitability of *Atyaephyra desmarestii* as a bioindicator of the effects of
3 hypoxic events on temperate freshwater systems, we investigated the respiratory responses of this
4 shrimp to increasing temperatures and progressive hypoxia at 2 different salinities. This study,
5 based on laboratory measurements, clearly demonstrates that this species has a moderate to poor
6 oxygen regulatory ability and a high sensitivity to hypoxia. The respiratory response of the shrimp
7 to increasing temperatures with increasing M_{O_2} (low thermal regulation in oxygen uptake) was
8 observed within a wide thermal range (15 to 30°C), although the species seems to be vulnerable to
9 temperatures above 25°C in brackish water. This species copes best with hypoxia in brackish
10 water but only at low to moderate temperatures ($T \leq 20^\circ\text{C}$). In freshwater, its habitual habitat, the
11 ability of *A. desmarestii* to oxyregulate seems to be less temperature dependent. These features
12 suggest that the respiratory response of *A. desmarestii* could be used in short term stress bioassays
13 to test the consequences of climate change and pollution events which lead to increased hypoxia of
14 aquatic habitats.

15 Keywords

16 *Atyaephyra desmarestii*, hypoxia, model species, bioindicator, oxyregulation,
17 oxygen consumption

1 Introduction

18 Hypoxic and anoxic events in aquatic systems have increased worldwide as a
19 result of climate change (warming and altered precipitation patterns) and high
20 nutrient run-off from agriculture and urban sewages (eutrophication).

21 Freshwater habitats are prone to hypoxia and anoxia when temperatures increase
22 (Diaz and Breitburg, 2009). When coupled with organic matter inputs, these
23 increases in temperature can also accelerate the eutrophication process, which is
24 an oxygen demanding process (Diaz, 2001). Organic enrichment combined with
25 hypoxia typically engenders depauperate macrobenthic communities (Rakocinski,
26 2012). In addition, changes in rainfall patterns and reduced freshwater due to
27 human use are expanding the boundaries of the brackish water realm, which is
28 characterized by lower oxygen solubility than freshwater.

29 Although warmer waters may reduce hypoxic thresholds in aquatic
30 organisms (Vaquer-Sunyer and Duarte, 2011), most species can maintain
31 adequate oxygen uptakes at dissolved oxygen concentrations (DO) above 5 mg l⁻¹
32 (Vaquer-Sunyer and Duarte, 2008; Diaz and Breitburg, 2009). Crustaceans are
33 among the most sensitive aquatic taxa to hypoxia, as 50% of studied species show

34 sublethal effects at a DO below 3.2 mg l⁻¹ (Vaquer-Sunyer and Duarte, 2008,
35 2011). When the DO drops below this optimum level, many species require
36 physiological and behavioural adaptations to maintain satisfactory oxygen uptake
37 rates (Bridges and Brand, 1980; Wannamaker and Rice, 2000; Salvato et al.,
38 2001; Bernatis et al., 2007). However, these adaptations can be energetically
39 expensive, reducing the energy available for growth and reproduction (Das and
40 Stickle, 1994), or even insufficient (below a hypoxic threshold), since the
41 respiratory rates of the organisms become more dependent on the external DO
42 (Bridges and Brand, 1980; Hicks and McMahon, 2002). Organisms exposed to
43 hypoxic conditions are also less tolerant to environmental stresses (food
44 limitations, thermal stress, pollutants) than those exposed to normoxic water
45 (Cairns et al., 1975; Shimps et al., 2005; Paschke et al., 2010; Vaquer-Sunyer and
46 Duarte, 2011). Therefore, evaluating respiration rates and their dependence on DO
47 could be a very appropriate way of determining the potential effects of warming
48 and eutrophication on aquatic communities (Fränze, 2006).

49 Freshwater shrimp are easy to culture and are highly sensitivity to
50 pollutants, which makes them useful organisms for biomonitoring programs.
51 *Atyaephyra desmarestii*, which is present in both lotic and lentic habitats, is the
52 species with the widest geographical distribution among the three freshwater
53 shrimps of the Iberian Peninsula (García-Muñoz et al., 2009). *A. desmarestii* is a
54 large detritivore and herbivore crustacean, which plays an important role in the
55 food web of freshwater habitats (García-Berthou and Moreno-Amich, 2000). Due
56 to its sensitivity to mine acid drainage and metals, it is considered a valuable
57 model species in short term stress bioassays (Janssens de Bisthoven et al., 2006;
58 Pestana et al., 2007). *A. desmarestii* is relatively tolerant to changes in
59 temperature and to a lesser extent in salinity; however, it generally inhabits well

oxygenated waters, which suggests that the species is sensitive to low DO. In this study, the respiratory responses to increasing temperature (warming effects) and progressive hypoxia (eutrophication effects) were examined in a population from southwestern Spain to assess whether *A. desmarestii* is an appropriate candidate for testing the potential effects of climate change and pollution events on temperate freshwater systems.

2. Material and Methods

2.1. Shrimp collection and maintenance

Specimens of *A. desmarestii* were collected by hand net from a tributary of the Guadalete River (SW Spain) in November 2010. After a 24-h acclimation period to aquariums, animals were transferred from the original water to aerated freshwater (tap dechlorinated water) and brackish water (12 of salinity) obtained by dilution of sea water. These salinities represent the boundaries of the salinity range within which the species hyper-osmoregulate (unpublished data). For at least 1 (freshwater) or 3 (brackish water) weeks prior to the experiments, shrimps were maintained at a temperature of 20 ± 1 °C under natural light and dark conditions, and daily supplied with commercial dry flakes (TetraMin).

2.2. Oxygen consumption measurements

The oxygen consumption of individual shrimps was measured using Strathkelvin 1302 polarographic electrodes connected to Strathkelvin 782 dual-channel oxygen meter. Oxygen concentration versus time were recorded every 20 s in a computer. Experiments were carried out during daytime, in black opaque glass chambers of 8 to 20 ml, depending on shrimp sizes, which were maintained at constant temperature (± 0.1 °C). Tested temperatures were 15, 20, 25 and 30 °C. Although not significant differences were observed between short-term (1 to 2h)

85 acclimated and non acclimated shrimps, individuals used in experiments at 30 °C
86 remained exposed to this temperature during 1h prior to respiratory measures.
87 Experimental salinities were produced using artificial seawater (Instant Ocean,
88 Aquarium Systems, Mentor, OH). For each salinity-temperature combination, 18
89 to 25 shrimps were used. Mean ($\pm$ standard error) wet weights of shrimp used in
90 each salinity-temperature combination varied between 0.11 ± 0.01 (15 °C; 0.3 of
91 salinity) and 0.15 ± 0.01 g (30 °C; 0.3 salinity), whereas individual wet weights
92 did between 0.05 and 0.33 g (± 0.0001 g).

93 **2.3. Data Analysis**

94 Specific oxygen consumption rates (M_{O_2}) were estimated on the
95 continuous plots of DO versus time excluding the first ten minutes to avoid the
96 manipulation stress of shrimps. Short term oscillations were smoothed by fitting
97 measures to a third order polynomial curve (Figure 1). Smoothed values were
98 used to obtain mean M_{O_2} values, as a function of DO grouped into $0.5 \text{ mg O}_2 \text{ l}^{-1}$
99 intervals, for the whole range of DO covered by the experiment (≈ 7 to 0.5 mg O_2
100 l^{-1}). Since absolute M_{O_2} values were shrimp size-dependent, the oxyregulation
101 degree of each shrimp was estimated on the curve build with M_{O_2} expressed on a
102 relative basis. For comparative purpose among the different temperature-salinity
103 combinations, the M_{O_2} measured at 6 mg l^{-1} was considered to be equal to 100 and
104 the rest of specific rates were calculated relative to this reference value. In this
105 plot, completely oxygen-independent behaviour (constant M_{O_2} irrespectively of
106 DO = oxyregulation) is expected to yield a horizontal straight line; completely
107 oxygen-dependent behaviour (oxyconformity), a straight line with a slope equal to
108 1; and partial oxygen dependence should yield curve, the curvature of which
109 depends on the degree of dependence (Figure 1). For each tested specimen, the
110 degree of oxyregulation with progressive hypoxia was assessed as a “percent O_2

111 regulation” value (Hicks and McMahon, 2002). This value was computed as the
112 difference between the expected reduction in oxygen uptake for a total
113 oxyconform individual and the observed reduction (Figure 1). Namely, the degree
114 of oxyregulation was estimated at DO of 5 mg l⁻¹ (well-oxygenated water), 3 mg l⁻¹
115 (¹ ≈ hypoxic threshold for sublethal effects on crustaceans) and 1 mg l⁻¹ (severe
116 hypoxia). For each shrimp, an overall oxyregulation was also estimated at DO
117 between 6 and 0.5 mg l⁻¹.

118 Additionally, to test for differences in absolute M_{O2} values at the three
119 selected levels of DO (5, 3 and 1 mg O₂ l⁻¹), prior to statistical analysis, effects of
120 shrimp sizes on their M_{O2} were overcome by adjusting them to those of 0.12 g wet
121 weight specimen using the corresponding (salinity-temperature) potential least-
122 squares regression between the M_{O2} (mg O₂ g⁻¹ h⁻¹) and the wet weight (g) of
123 shrimps (González-Ortegón et al., 2010). The Q₁₀ between each pair of
124 temperatures differing in 5 °C (T_i and T_j) was estimated as (M_{O2j}/ M_{O2i})².

125 Statistical differences in percent O₂ regulation and absolute M_{O2} values at
126 each hypoxic level were tested by two-way ANOVAs (factors: salinity and
127 temperature). Since significant factor interaction existed, data sets were split by
128 temperatures and salinity to perform a further one-way (factor: temperature-
129 salinity interaction) ANOVA. Student-Newman-Keuls tests were used as a
130 method of post hoc comparison (Zar 2010). Additionally, for each temperature
131 and salinity combination, percent O₂ regulation and M_{O2} values under different
132 DO conditions were compared by using paired-sample t-tests. A 5% significance
133 level (P =0.05) was considered for all statistically tests. Since multiple paired-
134 sample t-tests were carried out, Bonferroni correction was applied to avoid an
135 increase of statistically significant differences; that is, an experimental P of 0.002

136 was considered equivalent to a corrected P of 0.05. Prior to statistical analyses
137 data were logged (M_{O_2}) or arcsine (% O_2 regulation) transformed to homogenize
138 variances.

139 **3. Results**

140 The M_{O_2} varied greatly between individuals for each salinity-temperature
141 combination, and there was an oxygen-dependent M_{O_2} trend irrespective of the
142 DO (Figure 2). Nevertheless, the degree of oxygen dependence showed some DO
143 related variation: although the shrimp's oxyregulation increased from well-
144 oxygenated water to moderate hypoxia, a decreasing trend was observed under
145 severe hypoxic conditions (Figure 3 and Table 1). We also found an interaction
146 between salinity and temperature that affected the shrimp's oxyregulation
147 capacity: shrimps exposed to brackish water displayed a more temperature-
148 dependent response with lower mean oxyregulation values under higher
149 temperature conditions (Figure 3). Owing to the high M_{O_2} variability between
150 individuals, these differences were only statistically significant under moderate
151 and severe hypoxic conditions (Figure 4). Concurrently, the number of individuals
152 (%) with depressed M_{O_2} (negative O_2 regulation values) was temperature
153 dependent under severe hypoxia for both salinities, with a higher number of
154 shrimps showing depressed M_{O_2} at 30°C (Figure 4). Furthermore, at the end of the
155 respiratory experiments ($DO < 0.3 \text{ mg } O_2 \text{ l}^{-1}$), mortalities of shrimps at 15°C (5%
156 in freshwater and 11 % in brackish water) were lower than at 30°C (79% and 74%
157 respectively).

158 When the complete DO range (6 to 0.5 mg l^{-1}) was considered, the overall
159 degree of oxyregulation varied between 9.6 % at 25°C in freshwater and 19.5 % at
160 15°C in brackish water, although differences were only statistically significant

161 between the latter maximum value and the rest (except for shrimps at 25°C in
162 brackish water). The percentage of shrimps showing poor (< 10%) oxyregulation
163 varied between 11.8 % at 15°C and 52.2 % at 30°C in brackish water, and
164 between 33.3% and 46.2% respectively in freshwater (Figure 2).

165 The size-corrected M_{O_2} values showed increased respiration rates versus
166 increased temperatures irrespective of the DO and salinity conditions (Figure 3),
167 with the exception of shrimps at 25 and 30°C in brackish water, which always had
168 similar M_{O_2} values (Q_{10} : 0.8-1.0). Indeed, in well oxygenated water (5 O₂ mg l⁻¹),
169 the mean M_{O_2} values of a 0.12 g shrimp resulted in a Q_{10} of 1.6 between 15°C and
170 30°C in freshwater and a Q_{10} of 2.1 between 15°C and 25°C in brackish water.
171 Under severe hypoxia (1 O₂ mg l⁻¹), the mean M_{O_2} values resulted in a Q_{10} of 1.7
172 both between 15°C and 30°C in freshwater and between 15°C and 25°C in
173 brackish water (Figure 3). Within each temperature-salinity combination, there
174 was always a significant DO-related decrease in M_{O_2} values, although for the
175 same change in DO the decreases in M_{O_2} were smaller between well oxygenated
176 water and moderate hypoxia than between moderate and severe hypoxia (Figure 4
177 and Table 1).

178 4. Discussion

179 The physiological characteristics of organisms generally respond to the
180 environmental changes before the populations and communities do (Barbieri,
181 2007). Due to this early response, the oxygen consumption of organisms could be
182 a useful tool for testing the effects of environmental changes that lead to lower
183 DO levels. Nevertheless, the M_{O_2} of aquatic organisms is very variable (Ghiretti,
184 1966; Mangum and Van Winkle, 1973; Vernberg, 1983; Pihl et al., 1991; Salvato
185 et al., 2001) and its effectiveness as a measure of environmental change in

hypoxia scenarios depends on the oxyregulation capacity of the monitored species. As expected for a species which normally lives in well-oxygenated freshwater, *Atyaephyra desmarestii* has a poorer oxyregulation capacity than species that live in habitats exposed to periodic hypoxic events (Bridges and Brand, 1980; McMahon and Wilkens, 1983; Vernberg, 1983; Salvato et al., 2001; Hicks and Mahon, 2002; Pascual and Drake, 2008; González-Ortegón et al., 2010). Moreover, the species shows limited thermal regulation of oxygen uptake within a wide thermal range. These two features suggest that the respiratory response of *A. desmarestii* could be monitored in short term bioassays to test the effects of environmental changes that lead to temperature-related changes in the water oxygenation.

The high variability of M_{O_2} between *Atyaephyra desmarestii* individuals is an almost universal feature of ectothermic invertebrates (Ghiretti, 1966; Vernberg, 1983; Salvato et al., 2001; Vilas et al., 2006). As this variability is due to both intrinsic and extrinsic factors, it can be partially avoided in future research by selecting a homogeneous (size, gender, season, habitat) group of individuals that best fits the aims of the study. Another potential limitation of this study is that the organisms were kept at 20°C and exposed directly (except in 30°C experiments) to respiratory chambers at the target temperatures. Thus, the trends reported here should be considered as a measure of the “acute” thermal regulation of M_{O_2} and oxy-independence under declining DO; that is, an experimental approach corresponding to the daily short-term changes in temperature and DO which are frequently observed in shallow aquatic habitats of temperate regions (Dalla Via, 1985; Arias and Drake, 1987; Pascual and Drake, 2008). However, the respiratory response of *A. desmarestii* obtained in this study might have been different if shrimps had been allowed to acclimatize to the progressively decreasing

212 temperature, as in the seasonal temperature trend in temperate regions. Long-term
213 acclimatization sometimes improves an organism's tolerance to environmental
214 change (Dalla Via, 1987ab), but unfavourable environmental conditions at or
215 above sublethal physiological limits may also lead to a poorer physiological
216 condition (Leroi et al., 1994; Hicks and Mahon, 2002; Vilas et al., 2009) and
217 consequently lower M_{O_2} and oxyregulation abilities. In any case, the
218 aforementioned limitations can be easily overcome by using control replicates in
219 the experimental bioassay design.

220 As the cost of osmotic regulation is usually minimal at isosmotic
221 conditions, shrimps exposed to osmotic stress should show an increase in
222 metabolic rate. The isosmotic point for *A. desmarestii* is around 17 salinity at
223 20°C (unpublished data) and lower oxygen consumption, at least for this
224 temperature, is expected for shrimps in brackish water than in freshwater.
225 However, non-salinity related differences in M_{O_2} and respiratory independence
226 were observed at 20°C irrespective of the DO. Furthermore, as previously
227 reported for the freshwater shrimp *Palaemonetes antennarius* (Dalla Via,
228 1987a,b), the respiratory behaviour of *A. desmarestii* exhibited stronger
229 temperature dependence in brackish water than in freshwater. That is, under
230 hypoxic conditions and at a moderate temperature (15°C), shrimps from brackish
231 water displayed a significantly higher M_{O_2} and oxyregulation than freshwater
232 shrimps; however, these differences were not significant at higher temperatures
233 (20-30°C). A similar decrease in oxygen independence with increasing
234 temperature has been found previously in crustaceans inhabiting brackish and sea
235 water (Taylor, 1976; Bridges and Brand, 1980; Dalla Via, 1985; Daoud et al.,
236 2007; Cerezo et al., 2009). In addition, a change in water temperature from 25 to
237 30°C did not increase the M_{O_2} of the *A. desmarestii* in brackish water, which

238 suggests that temperatures above 25°C may represent a physiological limit for this
239 species when exposed to 12 salinity (Van Donk and De Wilde, 1981; Hicks and
240 McMahon, 2002). For freshwater shrimps, however, a temperature above 25°C
241 seems to represent a physiological limitation only under severe hypoxic
242 conditions. Therefore, in a climate change scenario of increased water temperature
243 and decreased DO (Vaquer-Sunyer and Duarte, 2008, 2011; Conley et al., 2009),
244 the expansion of the brackish water realm could represent an additional handicap
245 for the study species.

246 Aquatic organisms, such as fish and crustaceans, compensate for hypoxia
247 by increasing ventilation (Pihl et al., 1991; Maltby, 1995; Wannamaker and Rice,
248 2000; Hicks and McMahon, 2002), which requires an extra supply of energy.
249 However, at severe hypoxic levels, specimens become less active because they are
250 energy-limited (Das and Stickle, 1994; Hervant et al., 1998; McMahon, 2001).
251 Irrespective of the water temperature, the maximum level of oxyregulation of *A.*
252 *desmarestii* occurred under moderate hypoxic conditions (Figure 3). After this
253 maximum, increased hypoxia always resulted in decreased oxyregulation.
254 Nevertheless, as previously found for other species (Vaquer-Sunyer and Duarte,
255 2011), oxyregulation began to decrease at a higher DO threshold in warmer water,
256 especially at salinities of 12. Concurrently, the decreases in M_{O_2} were stronger
257 under hypoxic conditions than in well oxygenated water (Table 1). Thus, it seems
258 reasonable to hypothesize that the maximum level of oxyregulation of *A.*
259 *desmarestii* was a consequence of its behavioural changes (increased ventilation)
260 to compensate for the decrease in DO, whereas the posterior decline in
261 oxyregulation and M_{O_2} suggests that further adjustments of the respiratory pump
262 only served to supply its own increased oxygen demand (Bridges and Brand,
263 1980). Under this assumption, *A. desmarestii* may survive under hypoxic

264 conditions in brackish water but only at low to moderate temperatures ($T \leq 20^{\circ}\text{C}$);
265 however, in freshwater, its habitual habitat, the oxyregulation capability of *A.*
266 *desmarestii* seems to be less temperature dependent.

267 Anthropogenic habitat alterations may contribute to placing previously
268 well adapted native species at a competitive disadvantage to invasive species.
269 Under a scenario of increased hypoxic stress in aquatic habitats (eutrophication
270 and climate warming), the ability to maintain the M_{O_2} independently of declining
271 DO values may become an essential adaptation of organisms for inhabiting the
272 resulting oxygen-depleted environments (Bridges and Brand, 1980; Diaz and
273 Rosenberg, 1995). Thus, some native species could become more vulnerable to
274 competitive displacement by less oxygen-sensitive invader species (Byers, 2002;
275 Jewett et al., 2005; González-Ortegón et al., 2010). Despite its sensitivity to
276 hypoxia, *A. desmarestii*, whose original distribution was mainly freshwater
277 habitats from the Mediterranean Basin and the Middle East (García-Muñoz et al.,
278 2009), has successfully invaded northwestern Europe (Van den Brink and Van der
279 Velde, 1986; Straka and Špaček, 2009). Global warming could have contributed
280 to this northward displacement of the species because colder waters tend to have
281 higher DO. The wide network of artificial channels for inland shipping that
282 crosses the northwestern European countries has also facilitated the expansion of
283 the species to new river basins.

284 **5. Conclusions**

285 This study evidences that hypoxia does have a significant effect on the oxygen
286 consumption and oxyregulation level of *A. desmarestii* and provides valuable
287 information on the patterns of these responses. Furthermore, the shrimp's
288 sensitivity to the water oxygenation level and its currently wide geographical

289 distribution suggest that *A. desmarestii* is a good candidate for short-term
290 bioassays that aim to assess the consequences of climate change and pollution
291 events on temperate freshwater ecosystems.

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461 Figure Legends

462 **Fig. 1** Graphical examples of experimental data smoothing (third order polynomial curve fitting)
463 and oxyregulation measures of *A. desmarestii* (differences between experimental M_{O_2} values and
464 those expected in a total osmoconform shrimps)

465

466 **Fig. 2** Relative specific oxygen consumption rates of *A. desmarestii* shrimps exposed to different
467 combinations of temperature and salinity versus oxygen concentration inside the respiratory
468 chamber. Figures in the right bottom corner of each plot represent mean ($\pm$ SE) oxyregulation value
469 and percent of shrimps with poor ($< 10\%$) oxyregulation; lowercase letters, mean statistically
470 homogeneous values

471

472 **Fig. 3** For each temperature-salinity combination, mean values of shrimps' oxygen independence
473 versus oxygen concentration inside the respiratory chamber. Vertical lines indicate oxygen
474 concentrations at which the respiratory variables were statistically tested

475

476 **Fig. 4** Box plots of specific oxygen consumption rates (above), oxy-independence (middle) and
477 percent of shrimps, *A. desmarestii*, with depressed oxygen consumption (bottom) when they were
478 exposed to different temperature, salinity and oxygen concentration inside the respiratory chamber.
479 Lowercase letters indicate mean statistically homogeneous values; point-dashed lines, expected
480 values in perfect oxyconform shrimp

Table 1 For each temperature-salinity combination, changes in specific oxygen consumption rates (M_{O_2}) and oxy-independence (mean $\pm$ SE) of a 0.12 g wet weight *A. desmarestii* when exposed to different Cw_{O_2} conditions

Temp.-Salinity	Decrease in M_{O_2}		Change in % oxy-independence	
	5 to 3 $mg\ l^{-1} O_2$	3 to 1 $mg\ l^{-1} O_2$	5 to 3 $mg\ l^{-1} O_2$	3 to 1 $mg\ l^{-1} O_2$
15 °C - 0.3	127.8 $\pm$ 9.1 *	195.4 $\pm$ 11.3 *	9.2 $\pm$ 1.2 *	-4.5 $\pm$ 2.0
20 °C - 0.3	154.7 $\pm$ 15.3 *	254.6 $\pm$ 114.6 *	10.0 $\pm$ 1.1 *	-6.5 $\pm$ 1.1 *
25 °C - 0.3	247.5 $\pm$ 12.9 *	364.9 $\pm$ 14.4 *	7.5 $\pm$ 1.0 *	-5.9 $\pm$ 1.2 *
30 °C - 0.3	282.6 $\pm$ 24.7 *	488.5 $\pm$ 22.3 *	9.8 $\pm$ 1.4 *	-8.7 $\pm$ 1.8 *
15 °C - 12	93.9 $\pm$ 10.7 *	191.5 $\pm$ 16.9 *	14.8 $\pm$ 1.2 *	-1.6 $\pm$ 2.6
20 °C - 12	139.7 $\pm$ 15.6 *	263.6 $\pm$ 20.5 *	13.0 $\pm$ 1.1 *	-8.3 $\pm$ 2.9
25 °C - 12	241.6 $\pm$ 22.8 *	428.1 $\pm$ 20.5 *	10.5 $\pm$ 1.4 *	-6.7 $\pm$ 2.4 *
30 °C - 12	228.5 $\pm$ 22.0 *	445.9 $\pm$ 29.5 *	8.7 $\pm$ 1.3 *	-7.7 $\pm$ 2.9

*, $P < 0.05$ in paired-sample t-tests