**Additive effects of temperature and infection with an acanthocephalan
parasite on the shredding activity of *Gammarus fossarum* (Crustacea:
Amphipoda): the importance of aggregative behavior**

Temperature, parasitism and shredder role

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Keywords: global change, rising temperatures, thermal stress, keystone species, leaf litter decomposition, freshwater ecosystem, gammarid, trophic ecology, stressor, cumulative effects

Type of paper: Primary Research Article

Abstract

Climate change can have critical impacts on the ecological role of keystone species, leading to subsequent alterations within ecosystems. The consequences of climate change may be best predicted by understanding its interaction with the cumulative effects of other stressors, although this approach is rarely adopted. However, whether this interaction is additive or interactive can hardly be predicted from studies examining a single factor at a time. In particular, biotic interactions are known to induce modifications in the functional role of many species. Here, we explored the effect of temperature on leaf consumption by a keystone freshwater shredder, the amphipod *Gammarus fossarum*. This species is found at high densities in the wild, and relies on aggregation as an anti-predator behavior. In addition, gammarids regularly harbor acanthocephalan parasites that are known to induce multiple effects on their hosts, including modifications on their functional role. We thus assessed the cumulative effect of both intra-specific interactions and parasitism. Consumption tests were conducted on gammarids, either naturally infected with *Pomphorhynchus tereticollis* or uninfected, feeding alone or in groups. Our results show that increased temperatures induced a significant increase in consumption, but only to a certain extent. Interestingly, consumption at the highest temperature depended on amphipod density: whereas a decrease was observed for single individuals, no such effect on feeding was observed for individuals in groups. In addition, infection by acanthocephalan parasites per se significantly negatively impacted the shredding role of gammarids. Overall, the combined effects of parasitism and temperature appeared to be additive. Thus, future studies focusing on the impact of climate change on the functional role of keystone species may benefit from a multimodal approach under realistic conditions to derive accurate predictions.

Introduction

The ability to make accurate predictions about the consequences of climate change on ecosystem functioning and services depends for a large part on our understanding of how changes in temperature can interact with other sources of ecological disturbance, including biotic and abiotic factors. In this context, the impact of climate change on the functional role of keystone species deserves particular attention. By definition, keystone species play a major role in the structure of communities and ecosystem functioning (Mills *et al.*, 1993; Jordán, 2009). Several studies have shown that climate change can affect the behavior of keystone species (Sanford, 1999; Zamani *et al.*, 2006; Pincebourde *et al.*, 2008; Kidawa *et al.*, 2010; Englund *et al.*, 2011; Luque *et al.*, 2014; Hamilton *et al.*, 2015), with potential consequences at the level of ecosystems. However in terms of predictions, an important issue is whether the effect of temperature on keystone species acts in synergy or antagonistically with other biotic and abiotic stressors (Crain *et al.*, 2008; Darling & Côté, 2008). In particular, meta-analyses suggest that the effect of climate change on organisms could lead to unpredictable changes in inter and intra-specific interactions (Tylianakis *et al.*, 2008; Rosenblatt & Schmitz, 2014). Indeed, biotic interactions can strongly affect the functional role of species within ecosystems. Hawlena *et al.* (2012) showed, for instance, that the effectiveness of plant-litter decomposition by grasshoppers was reduced under predation risk. Biotic interactions could then strongly influence the effect of climate change on organisms and their functional roles (Gilman *et al.*, 2010), although the shape of such complex interactions cannot be predicted from studies conducted on single species.

Parasitic infections constitute one of the most common biotic interactions, considering that most living organisms represent a potential host for many parasites (Poulin & Morand, 2000). Furthermore, parasites often constitute a stressor for these organisms. Parasites are

well known to impact their hosts in multiple ways, potentially affecting their functional role (Thomas *et al.*, 1999; Hernandez & Sukhdeo, 2008). For example, parasitic infections can alter the diet of hosts foraging opportunistically (Médoc *et al.*, 2011), or alter food web structure by diverting predators from host preys as a consequence of parasite-induced behavioral changes in hosts (Sato *et al.*, 2012). In addition, parasites are themselves highly affected by changes in temperature (Marcogliese, 2001; Morley & Lewis, 2014). Although parasites and global warming are likely to have cumulative effects on the role of key species within ecosystems (Lafferty & Kuris, 1999; Labaude *et al.*, 2015a), it is not clear whether such effects would be additive or interactive.

Gammarid amphipods are widespread and often abundant throughout diverse aquatic habitats (Macneil *et al.*, 1997; Piscart *et al.*, 2009). They are regarded as key species within freshwater food webs as they constitute an important trophic resource for many species (Degani *et al.*, 1987; Friberg *et al.*, 1994), and are themselves a major predator for many invertebrate species (Macneil *et al.*, 1997; Kelly *et al.*, 2002), thus modulating the composition of freshwater macroinvertebrates community (Kelly *et al.*, 2006). More to the point, gammarids have important scavenger and shredder roles (Macneil *et al.*, 1997; Felten *et al.*, 2008), controlling the leaf litter breakdown in certain ecosystems (Piscart *et al.*, 2009; Constable & Birkby, 2016) and maintaining water quality (Maltby *et al.*, 2002). They are therefore of significant economic and ecological importance, as water quality plays a crucial role in the emergence of water-borne diseases, ecosystem dysfunction and loss of biodiversity (Klaphake *et al.*, 2001). However, the functional role of gammarids as shredders has been shown to depend upon several parameters including both biotic interactions, such as the presence of other gammarid species (Piscart *et al.*, 2011; Constable & Birkby, 2016) or predators (Åbjörnsson *et al.*, 2000), and abiotic parameters, such as microhabitat characteristics (Felten *et al.*, 2008) or the presence of pollutants (Dedourge-Geffard *et al.*,

2009; Andreï *et al.*, 2015). Recently, it has been demonstrated that the consumption of leaves by *Gammarus pulex* amphipods tended to increase, though not linearly, with rising temperatures (Foucreau *et al.*, 2016; see also Pellan *et al.*, 2015). *Pomphorhynchus tereticollis*. Although this population is known to contain only *G. fossarum* gammarids (Laguerre *et al.*, 2014), we conducted genetic analyses on 36 individuals to control for the presence of the cryptic, closely-related species *G. pulex*. Individuals from both sexes and both infection status (uninfected or naturally infected with *P. tereticollis*) were collected.

Gammarids are the intermediate host for *P. tereticollis* parasites, in which they develop successively in two distinct larval stages: acanthella and cystacanth stages. At this second stage, which is infective to fish final hosts, parasites induce a whole range of modifications in their gammarid hosts, including changes in behavior (Tain *et al.*, 2006; Perrot-Minnot *et al.*, 2012) or immune system (Cornet *et al.*, 2009), although, to our knowledge, the effect of this parasite species on food consumption by gammarids has not been investigated. Therefore, gammarids infected with the cystacanth stage of the parasite were used in this experiment. Thanks to their yellow-orange coloration, cystacanths are clearly visible through the translucent cuticle of gammarids, allowing a preliminary selection of infected individuals directly upon collection in the field.

Acclimatization of animals

Gammarids collected in the field were immediately placed in acclimatization in the laboratory for ten days. They were maintained in groups of several dozens of individuals, in boxes (10.5 × 16 cm) filled with an oxygenated mix of water from the Norges river and dechlorinated, UV-treated tap water. All amphipods were fed *ad libitum* with conditioned elm leaves, and

maintained under a 12:12 light:dark cycle regime. Groups were randomly assigned to one temperature (10, 14 or 18°C), for both the acclimatization period and the subsequent consumption test. This range of temperatures was chosen based on naturally fluctuating conditions in the habitat of *G. fossarum* (Pöckl *et al.*, 2003), current temperatures in Burgundy, and predictions for 2100 (Gunn & Crumley, 1991; Rowell, 2005). Boxes containing amphipods were placed in water baths, allowing all three temperatures to be tested simultaneously. The surrounding water was constantly recirculated through a temperature control device (TANK TK-1000 Chiller, Teco US). Water temperature was monitored daily.

Individual food consumption

Before each test, individuals were checked under a binocular microscope to identify gammarids containing no visible parasite (controls) and gammarids containing *P. tereticollis* cystacanths. Single individuals were placed into individual glass dishes (six cm diameter), at acclimatization temperature (10, 14 or 18°C), and starved for 24 hours. Individuals were then provided a leaf disc of known dry weight, and were allowed to feed for 24 hours. Three replicates were conducted at each of the three temperatures (one replicate per day). For each replicate, paired glass dishes at each temperature were left free of gammarids, serving as control for the natural deterioration of leaves. A total of 145 males and 156 females were used for this experiment.

Leaf discs were prepared before each test. Humidified elm leaves were sterilized at 120°C for 20 minutes, and cut into 1.5 cm diameter discs using a cork borer. Discs were then dried at 50°C until they stopped losing water, as estimated by weighing leaves regularly during a pre-test. This took three hours for the 1.5 cm diameter disks. Disks were then

weighed to the nearest tenth of milligram, and re-hydrated for 24 hours before the test. After the test, each leaf disc was dried again for three hours and weighed.

Food consumption in microcosms

After acclimatization (at 10, 14 and 18°C), gammarids were placed in groups of ten individuals, either all infected or all uninfected (based on visual inspection under a binocular microscope), in larger glass dishes (10 cm diameter) containing a few stones under which they could hide. Apart from their infection status, individuals were randomly chosen, such that the sex-ratio was variable between groups. Gammarids were starved for only five hours, to avoid cannibalism. They were then provided with pieces of elm leaves of known dry weight, and were allowed to feed for 48 hours. Several glass dishes at each temperature were free of gammarids, serving as controls for the natural deterioration of leaves. A total of 270 infected gammarids were used (27 groups), while 390 uninfected gammarids were tested (39 groups). Five replicates were conducted at each of the three temperatures (one replicate per day).

Elm leaves were prepared and weighed as described before, except that they were dried at 50°C for a longer time. Indeed, due to larger amount of leaves, as well as their bigger size, pre-tests showed that at least six hours were necessary to dry them completely. Batches of approximately 200 mg (dry weight) were provided to gammarids.

Measurements and dissections

After each test, all individuals were sexed based on the size and shape of their first and second pairs of gnathopods, which present a sexual dimorphism in amphipods (Hume *et al.*, 2005).

Following Bollache *et al.* (2000), body size was measured as the height of the fourth coxal plate using a microscope and Lucia G 4.81 software. Gammarids were then dissected to determine their infection status (presence or absence of parasites), parasite developmental stage and parasite species based on morphological identification (as the acanthocephalans *P. laevis*, *Polymorphus minutus* and *Echinorhynchus truttae* can also be found in the Norges river, although at much lower frequencies than that of *P. tereticollis*). Individuals were categorized as “parasitized” when they were harboring at least one *P. tereticollis* cystacanth stage, and “control” if no parasite was found. Individuals harboring only acanthella (immature stage) or harboring cystacanths from other acanthocephalan species were excluded from the data set. Individuals which died during the tests were also removed from the data set.

Although consumption is usually expressed on a dry weight basis (Foucreau *et al.*, 2013a; Dianne *et al.*, 2014; Schmidlin *et al.*, 2015a), the need to dissect individuals made this impossible. Instead, linear measures of gammarids were recorded (i.e. the height of the fourth coxal plate). In addition to gammarids used in consumption tests, we also measured 137 uninfected *G. fossarum* from the Norges river (68 males and 69 females). Those individuals were then dried in a 50°C chamber during 64 hours, and immediately weighed. The relationship between the height of the fourth coxal plate and the dry weight of individuals was then calculated for this population, such that the size of individuals could be considered in the calculation of individual consumption.

Data analyses

Estimation of food consumption

The quantity of food (Q_i , in mg) consumed for each individual or group of individuals was calculated as follows:

$$Q_i = MB_i^* - MA_i$$

where (MA_i) represents the mass of the disc or batch of leaves after consumption and (MB_i^*) the mass before, corrected to take into account the quantity of food that was lost due to natural deterioration. To estimate this value, we calculated for each replicate the mean ratio between MCA, the mass of control leaves (leaves left without gammarids) measured at the end of the experiment, and MCB, the mass of control leaves measured at the beginning of the experiments. We then multiplied these estimates of natural deterioration by the mass of disc or batch of leaves after consumption (MB_i), such that:

$$Q_i = .$$

The relationship between the wet mass and the height of the fourth coxal plate was found to follow a linear relationship ($R^2 = 0.71$, $P < 0.0001$) that did not differ between males and females. Thus, the size of gammarids was considered in the estimation by dividing Q_i with the height of the coxal plate for individual consumption, or by the mean value for all remaining individuals in each group for microcosm consumption.

Individual food consumption

Food consumption data were ($\log +1$) transformed in order to normalize residuals.

Transformed data were analyzed with a linear model, considering temperature (as an ordinal variable), sex, status (parasitized vs control), and their interactions, and including replicate as a random factor. Non-significant interactions were removed from the model. Tukey HSD

post-hoc tests were then used to compare food consumption differences between pairs of temperatures.

To analyze the strength of the effects of both temperature and parasitism on food consumption, Cohen's *d* effect sizes (Cohen, 1988) and their 95% confidence intervals were calculated for differences between parasitized and control individuals at each temperature and for each sex, and between all the temperatures for each status and for each sex. Cohen's *d* is a scale-less parameter used to represent the size of the effect, such as a difference between two groups, and the direction of this difference (Nakagawa & Cuthill, 2007). Cohen's *d* is calculated using the means and standard deviations of those groups, and confidence intervals are calculated with a bootstrap technique.

Food consumption in microcosms

After controlling for normality of model residuals using Q-Q plots, microcosm consumption was also analyzed using a linear model, with temperature, group status (parasitized vs control) and group sex-ratio as fixed factors, and replicate as a random factor. Non-significant interactions were removed from the model. Tukey HSD post-hoc tests were used to compare food consumption differences between pairs of temperatures. Cohen's *d* were then used to analyze the effect sizes, as described before.

Models were performed using JMP version 10.0.0 software (SAS Institute, Cary, NC, U.S.A.) and Cohen's *d* effect sizes were calculated using R version 3.1.1 software (R Foundation for Statistical Computing).

Results

Individual food consumption

The three single factors were significantly affecting gammarids individuals food consumption (temperature: $F_{2, 294} = 3.96$, $P = 0.02$; sex: $F_{1, 294.5} = 5.36$, $P = 0.02$ and infection status: $F_{1, 294.3} = 33.22$, $P < 0.0001$), whereas no interaction was significant. Overall, food consumption was higher for males compared to females, and in control individuals compared to parasitized ones (Fig. 1). The overall consumption tended to increase between 10°C and 14°C, but then decreased at 18°C (Fig. 1). Tukey's range tests showed that the difference was only significant between 14°C and 18°C.

Cohen's d confirmed that consumption was more important for control individuals compared to parasitized ones, with all differences being significant except between females at 10°C and 14°C, for which there was however a clear trend (Fig. 2). Comparisons between temperatures showed that parasitized individuals, males and females, consumed significantly more food at 14°C compared to 18°C, with a marginally-significant decrease between 10°C and 18°C. Uninfected males also ate more at 14°C compared to 18°C, while all other differences were non-significant, especially in females (Fig. 3).

Food consumption in microcosms

Mortality during the test occurred for 15 groups out of 66, with no difference between parasitized and control groups (Chi-squared test: 3.34, d.f. = 2, $p = 0.16$). Thus, all the groups were kept in the model.

The consumption of individuals in microcosms was significantly greater for control groups than the parasitized groups ($F_{1, 58.21} = 42.38$, $P < 0.0001$, Fig. 4). There was a positive, but not significant relationship between food consumption and the proportion of males ($F_{1, 59.78} = 3.19$, $P = 0.079$). Temperature also significantly influenced food consumption ($F_{2, 58.5} = 5.75$, $P = 0.005$, Fig. 4), and Tukey's HSD post hoc tests showed that differences in consumption were significant only between 10°C and the two other temperatures.

Cohen's d confirmed that parasitized groups consumed significantly less food at all temperatures, compared to control groups (Fig. 2), and that these differences were stronger than those observed during individual tests (Fig. 2). Comparisons between temperatures showed that the pattern was different between parasitized and control groups: while consumption increased with temperature for parasitized groups, there was no difference in consumption for control groups between 14°C and 18°C (Fig. 3).

Discussion

Our results clearly show that temperature had an important impact on the shredding role of gammarids, with a positive effect between low and medium temperatures, followed by either a stagnation or a diminution of consumption by gammarids at the highest temperature. A negative effect of the parasite was found on gammarid consumption at all temperatures, suggesting an overall additive effect of temperature and parasitism. Feeding alone or in a group was also found to be of significant importance for food consumption by gammarids, influencing response to temperature.

Assuming that infection is costly and increases gammarids metabolism (Labaude *et al.*, 2015b), we would have expected infected gammarids to increase leaf consumption in compensation. However, in accordance with Médoc *et al.* (2011), parasitism was found to negatively affect the consumption of leaves by gammarids in all the situations that we tested. *P. tereticollis* cystacanths are known to alter the behavior of their hosts, possibly increasing the probability of trophic transmission (Perrot-Minnot *et al.*, 2007). A decrease in food consumption could thus be linked to such manipulation, although Dianne *et al.* (2014) found that gammarids infected by the acanthella stage (i.e. not infective for the definitive host) of the closely-related *P. laevis* also consumed less food than uninfected individuals. More likely, the negative effect of parasitism could result from some stress induced by the parasite. Indeed, although the fitness of *P. laevis* is highly dependent on the survival of its intermediate host before trophic transmission occurs, acanthocephalan-infected gammarids have a higher mortality than uninfected ones under laboratory conditions (Cornet & Sorci, 2010; Labaude *et al.*, 2015b). Most studies have reported a lower resistance of female gammarids to different stress (pollutants, hypoxia, salinity, temperature: McCahon & Pascoe, 1988; Hoback & Barnhart, 1996; Sornom *et al.*, 2010), potentially explaining the lower food consumption of

females compared to males, relative to their size, as observed in our study. Several other investigators have reported sex-related differences in food consumption by amphipods, but, contrary to our study, in most cases, food consumption was lower in males (Foucreau *et al.*, 2013b, 2014; Pellan *et al.*, 2015).

As expected, temperature affected gammarids food intake, with significant effects on leaf consumption in all conditions of infection and aggregation. An increase in temperature is known to increase metabolic rate in ectothermic species (Brown *et al.*, 2004), thus leading to higher energy requirements. Accordingly, and as observed in previous studies (Coulaud *et al.*, 2011; Pellan *et al.*, 2015; Schmidlin *et al.*, 2015a), leaf consumption increased between low and medium temperatures in the present study. However, at the highest temperature, food consumption dramatically decreased in most single individuals. Many parameters linked to the metabolic rate of gammarids, such as locomotor activity (Issartel *et al.*, 2005; Maazouzi *et al.*, 2011), oxygen consumption (Issartel *et al.*, 2005; Foucreau *et al.*, 2014), ventilatory activity (Issartel *et al.*, 2005) or glycogen content (Maazouzi *et al.*, 2011; Foucreau *et al.*, 2014), are known to first increase with temperature to a certain extent, before decreasing. Maazouzi *et al.* (2011) found, in the case of glycogen content, that such a decrease is tightly linked with thermal tolerance in *G. pulex*. The decrease in food consumption observed in the present study could thus be associated with thermal stress occurring at temperature well above the thermal optimum for gammarids. Considering that the thermal optimum for *G. fossarum* is approximately 11.5°C (Pöckl & Humpesch, 1990), with potential variations depending on the geographical origin of the population (Foucreau *et al.*, 2013b, 2014), it is not surprising that a temperature of 18°C would induce a non-lethal thermal stress, with consequences on food consumption. Similarly, Schmidlin *et al.* (2015b) observed a slightly reduced feeding activity of *G. fossarum* above 16°C, while that of males *Dikerogammarus villosus* decreased between 20°C and 25°C (Pellan *et al.*, 2015).

The effect of temperature on *G. fossarum* consumption was dependent on whether gammarids were feeding alone or in groups. Indeed, although a marked decrease was observed in lone individuals (apart from uninfected females) at the highest temperature, no such effect was observed in individuals feeding in groups. Gammarids are however a gregarious species, often present at high density in the wild, and relying on aggregation as an antipredator behavior (Durieux *et al.*, 2012). Our results thus suggest that experimental studies addressing the influence of climate change on keystone species may benefit from measuring their behavior under realistic conditions, i.e. as close as possible to natural conditions. Maintaining individuals in isolated conditions could actually result in a supplementary stress, especially at the highest temperature, at which a thermal stress is suspected. Moreover, the addition of a third stressor, parasitism, could contribute to explain why consumption decreased at the highest temperature in infected isolated females, whereas it only levelled off in uninfected ones. Thus, although several studies investigated food consumption by gammarids in isolated individuals (Bundschuh *et al.*, 2011; Foucreau *et al.*, 2013a, 2013b, 2016; Dianne *et al.*, 2014), their shredding efficiency could actually be highly dependent on the presence or absence of conspecifics. Results from studies measuring food consumption within groups (e.g. Coulaud *et al.*, 2011; Schmidlin *et al.*, 2015a, 2015b) might then be more reliable to extrapolate the influence of environmental factors on the functional role of keystone species such as amphipods.

Finally, the effect of high temperature on the shredder role of amphipods could be amplified by its direct effects on parasitic infection. Indeed, an increase in temperature can lead to an increase in both the prevalence and intensity of infection with parasites (Mouritsen *et al.*, 2005; Karvonen *et al.*, 2010; Schoebel *et al.*, 2011). Thus, through increasing the number of parasites within infected individuals, as well as the proportion of infected individuals, the overall effect of increased temperatures on the shredding efficiency of

gammarids might be even more pronounced than what was estimated in the present study, and therefore impact negatively the ecological sanitation role of gammarids in freshwater ecosystems. Other effects of increased temperature on the quality and distribution of plant species (Kelly & Goulden, 2008; Lenoir *et al.*, 2008) or on the impact of rival species, especially invasive ones (Dukes & Mooney, 1999; Walther *et al.*, 2009), may also affect the shredding efficiency of gammarids. Additionally, the interaction between parasitism and such invasive species (Prenter *et al.*, 2004) may add further complexity. Thus, although our approach analyzing the combined effects of abiotic and biotic factors (inter and intraspecific) provides a useful basis to understand the consequences of increasing temperatures on the shredding activity of detritivores, improving predictions might require further investigations to address this complexity. Future studies would benefit in accuracy by using approaches relying on long-term experiments and/or monitoring (Lepetz *et al.*, 2009).

Our results indicate that rising temperatures might induce an increase in the shredding efficiency of gammarids, at least to a certain extent. However, this effect might be countered by parasitism, which may depend on the prevalence and intensity of infection in natural populations. In addition, the intricate link between temperature, parasitism and other biotic interactions, such as conspecifics or individuals from other gammarid species, as well as combined effects on the shredder role of gammarids, highlight the difficulty in predicting the consequences of global change on the functional role of keystone species from studies based on a limited number of factors.

Acknowledgments

We thank Sébastien Motreuil and Ambre Salis for technical assistance, and the ANR (Grant ANR-13-BSV7-0004-01) for financial support. SL was supported by a Doctoral grant from the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche.

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Figures

Fig. 1. Individual *Gammarus fossarum* leaf consumption, according to infection status (P or C, respectively parasitized or control), their sex, and the temperature (10, 14 or 18°C).

Squares represent means for the quantity of leaf (in mg) consumed by individual gammarids during 24 hours, corrected by their size (height of the fourth coxal plate, in mm). Standard errors and sample sizes are indicated.

Fig. 2. Effect sizes of consumption differences between parasitized and control *Gammarus fossarum* maintained as individuals (males or females) or in microcosms, at each temperature.

Values above zero indicate that the consumption is higher for control individuals/groups compared to parasitized ones. The difference is significant when the bar does not overlap zero. Cohen's d effect sizes are represented with 95% confidence intervals.

Fig. 3. Effect sizes of the differences in consumption at the three experimental temperatures, for individuals (males and females, respectively m and f) and grouped (microcosms)

Gammarus fossarum, and for infection status (parasitized or control, respectively P or C).

Values above zero indicate that the consumption is higher for the first temperature cited, and dots under zero indicate higher consumption for the second temperature cited. The difference is significant when the bar does not overlap zero. Cohen's d effect sizes are represented with 95% confidence intervals.

Fig. 4. Leaf consumption of grouped *Gammarus fossarum* individuals in a microcosm, according to infection status (P or C, respectively parasitized or control), and the temperature (10, 14 or 18°C). Squares represent means for the quantity of leaf (in mg) consumed by average individuals in each group during 24 hours, corrected by their size (mean height of the fourth coxal plate of all individuals within the group, in mm). Standard errors and sample sizes are indicated.