



**LUDMILA ROCHA PENONI**

**ECOLOGICAL TRAITS OF TWO *HYALELLA* SMITH, 1874  
(CRUSTACEA: AMPHIPODA: HYALELLIDAE)  
MORPHOTYPES FROM SOUTHEASTERN BRAZIL**

**LAVRAS – MG  
2019**

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos Naturais em Ecossistemas Fragmentados e Ecossistemas, para a obtenção do título de Mestra.

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**CARACTERÍSTICAS ECOLÓGICAS DE DOIS MORFOTIPOS DE *HYALELLA*  
SMITH, 1874 (CRUSTACEA: AMPHIPODA: HYALELLIDAE) DO SUDESTE DO  
BRASIL**

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## RESUMO

Os crustáceos lacustres do gênero *Hyalella* são exclusivamente americanos, e apresentam ampla distribuição nas regiões neártica e neotropical. Por sua baixa capacidade de dispersão, muitas espécies são endêmicas, e acredita-se que atualmente o número de espécies conhecidas é muito inferior à realidade. Sessenta e três espécies são registradas para a América do Sul; destas, 28 ocorrem no território brasileiro. Para o estado de São Paulo, seis espécies são conhecidas, entretanto, as espécies encontradas no Parque Estadual da Serra do Mar – Núcleo Santa Virgínia (PESM – NSV) ainda não foram descritas. Além disso, os trabalhos com ecologia envolvendo as espécies brasileiras são escassos. Esta dissertação apresenta os resultados da pesquisa intitulada “Ecological traits of two *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) morphotypes from Southeastern Brazil”. O objetivo da pesquisa foi analisar os fatores do ambiente que permitem a coexistência de diferentes fases de vida e de dois diferentes morfotipos do gênero *Hyalella* através da divisão de nicho e as estratégias reprodutivas dos morfotipos, com base em dados de fecundidade. As coletas aconteceram em dois riachos no PESH – NSV em maio/2018. Os organismos coletados foram identificados em dois morfotipos (M1 e M2) e as variáveis do habitat físico foram utilizadas para observar a divisão de nichos entre morfotipos e estágios de vida. Nós encontramos 759 organismos (M1 = 356; M2 = 403). Análises de PCA confirmam a variação morfológica dos dois morfotipos e a sua divisão no espaço físico dos riachos. Através de análises de covariância, o M1 – organismos geralmente menores e com antenas menores – foi mais associado à ocorrência de margem escavada ao longo dos transectos, sendo essa variável negativamente estatisticamente relevante para a abundância deste morfotipo. Fêmeas e fêmeas ovígeras do M1 responderam bem a essa variável; machos do M1 se associaram positivamente à média do tamanho do substrato; juvenis do M1 não responderam a nenhuma variável do habitat físico. O M2, diferentemente, foi negativamente afetado pela cobertura vegetal. Fêmeas e fêmeas ovígeras do M2 responderam apenas a essa variável; machos do M2, além de cobertura vegetal, responderam positivamente à largura molhada. Juvenis do M2 tiveram preferência por locais com menor cobertura vegetal e maior disponibilidade de pedaços de madeira e arbustos. O número médio de ovos encontrados no marsúpio das fêmeas foi diferente entre os dois morfotipos, sendo que fêmeas do M2 carregam mais ovos. O volume médio do ovo e o volume médio de ovos carregados pelas fêmeas também variou entre os morfotipos, sendo que as fêmeas do M2 carregam ovos mais volumosos. Dessa forma, as hipóteses de que os dois morfotipos ocupam diferentes nichos, assim como há divisão de nichos entre diferentes fases de vida, foram confirmadas por nossas observações.

**Palavras-chave:** Habitat físico. Divisão de Nicho. Morfologia. Fecundidade

## ABSTRACT

The freshwater crustacean genus *Hyaella* is exclusive to the Americas, with a broad distribution on the nearctic and neotropical regions. Because of its low dispersion ability, many species are endemic, and it is believed that the current number of known species is much inferior to the reality. Sixty-three species are registered to South America, and 28 occur in Brazil. To this day, São Paulo state presents six known species, but none of them are registered to Parque Estadual da Serra do Mar – Núcleo Santa Virgínia (PESM – NSV). Furthermore, ecological research about Brazilian *Hyaella* species are rare. This dissertation presents the results of a research entitled “Ecological traits of two *Hyaella* Smith, 1874 (Crustacea: Amphipoda: Hyaellidae) morphotypes from Southeastern Brazil”. The objective of this research was to analyze the environmental factors that allow the coexistence of two *Hyaella* morphotypes through niche division and the reproductive strategies of the morphotypes, based on fecundity data. Collections were held in May/2018 at PESM – NSV. Collected organisms were identified in two morphotypes (M1 and M2), and the physical habitat variables were used to observe niche division between morphotypes and life stage categories. We found 759 organisms (M1 = 356; M2 = 403). Principal Component Analysis confirmed the morphological differences between morphotypes and their division on the physical space of the streams. Covariance analysis were used to identify the physical habitat variables that most relate to each morphotype and life stage category. Morphotype 1 organisms – usually small bodied, with smaller antennas – were negatively associated to the occurrence of boulders along the transects. Females and ovigerous females were closely related to this variable; males were positively affected by mean substrate size; and juveniles did not respond to any physical habitat variable. Morphotype 2 organisms, on the other hand, were negatively affected by canopy coverage. Females and ovigerous females were dependent only upon this variable; males responded negatively to canopy coverage and positively to wetted width; and juveniles displayed preference to areas with less canopy coverage and more availability of brush and small wood debris. The mean number of eggs in the ovigerous female’s marsupium differed between morphotypes, with M2 females bearing more eggs. Mean egg volume and mean volume of eggs in the marsupium also differed between morphotypes, with M2 females carrying more voluminous eggs. In summary, the hypothesis that the two morphotypes and the different life stages occupy different niches were endorsed by our results

**Keywords:** Physical habitat. Niche Division. Morphology. Fecundity.

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**PRIMEIRA PARTE**

## 1 INTRODUÇÃO

*Hyaella* Smith (1875) é um gênero de crustáceos anfípodos exclusivamente americano. Distribuído ao longo de todo o território neotropical e neártico, este gênero é composto por organismos dulcícolas, com representantes epígeos e hipógeos (BUENO; RODRIGUES; ARAÚJO, 2014; CARDOSO et al., 2014; RODRIGUES; BUENO; FERREIRA, 2014; RODRIGUES, 2016).

A grande distribuição geográfica do gênero em relação à sua baixa capacidade de dispersão (SWASON, 1984) resulta em uma alta riqueza de espécies endêmicas a pequenos cursos d'água. Até o momento, mais de 70 espécies de *Hyaella* foram descritas (BASTOS-PEREIRA; OLIVEIRA; FERREIRA, 2018; SEREJO; SIQUEIRA, 2018). Sabe-se que este número está subestimado; para algumas localidades, como o Lago Titicaca por exemplo, coletas apontam para diversas espécies ainda não descritas (GONZÁLEZ; WATLING, 2003; ADAMOWICZ ET AL. 2018).

A América do Sul concentra a maior riqueza de espécies conhecidas para o gênero, com o registro de ocorrência de 63 espécies (COLLA; CÉSAR, 2015; SOUCEK et al., 2015; RODRIGUES, 2016; ALONSO; JAUME, 2017). Ao todo, 28 espécies ocorrem no Brasil, sendo o Rio Grande do Sul o estado com maior riqueza registrada (RODRIGUES et al., 2017; STRECK et al., 2017; BASTOS-PEREIRA; OLIVEIRA; FERREIRA, 2018; SEREJO; SIQUEIRA, 2018). Há registro de *Hyaella* apenas para as regiões sul e sudeste do país (BUENO; RODRIGUES; ARAÚJO, 2014; CARDOSO et al., 2014; RODRIGUES; BUENO; FERREIRA, 2014; RODRIGUES, 2016; SEREJO; SIQUEIRA, 2018).

Seis espécies ocorrem no estado de São Paulo (CARDOSO; BUENO; FERREIRA, 2011; RODRIGUES; BUENO; FERREIRA, 2014): *H. caeca* Pereira, 1989; *H. dielaii* Pereira, 2004; *H. epikarstica* Rodrigues, Bueno e Ferreira, 2014; *H. meinerti* Stebbing, 1899; *H. spelaea* Cardoso e Bueno, 2011; e *H. warmingi* Stebbing, 1899 (González e Watling, 2003; Cardoso, 2013). Destas, quatro são subterrâneas e apresentam ou não características troglomórficas. *Hyaella caeca* foi a primeira espécie troglóbia a ser encontrada em território brasileiro, e o único trabalho sobre ela é o artigo de descrição (PEREIRA, 1989). Encontrada no mesmo município que *H. caeca*, mas em cavernas diferentes, *H. epikarstica* apresenta diversas adaptações para a vida subterrânea; seu nome é baseado no ambiente em que é encontrada, o epicarste (RODRIGUES; BUENO; FERREIRA, 2014). *Hyaella warmingi* ocorre na Gruta Mirasol, em Mirasol, na Gruta do Paraná, em Altinópolis, e na Gruta Jane

Mansfield, em Iporanga, todos no estado de São Paulo (CARDOSO, 2013). Por fim, *H. spelaea* tem como localidade tipo a Gruta da Toca, no município de Itirapina, centro-norte do estado (CARDOSO; BUENO; FERREIRA, 2011).

O único trabalho com *H. dielaii* é a sua descrição. *Hyaella meinerti* tem ocorrência registrada para o estado de São Paulo (23°20' S, 46°22' W) (GONZÁLEZ; WATLING, 2003), mas os autores não especificam uma localidade exata.

Contrariando a regra geral de que espécies de *Hyaella* se restringem a pequenas regiões geográficas, tanto *H. warmingi* quanto *H. meinerti* estão amplamente distribuídas, sendo que estas ocorrências devem ser revistas. Além de ambas espécies ocorrerem em São Paulo, a primeira tem registros para localidades na Venezuela e no Peru; e a segunda tem como localidade tipo Lagoa Santa, em Minas Gerais (GONZÁLEZ; WATLING, 2003; CARDOSO, 2013). Por se tratar de espécies com trabalhos de descrição muito antigos, e como são conjuntos de características que determinam a identificação de espécies, estes registros de ocorrência devem ser observados com cautela, sendo necessária uma revisão detalhada.

São poucos os trabalhos ecológicos envolvendo espécies de *Hyaella* no Brasil. Os únicos com ecologia de comunidades são os que estudaram as espécies simpátricas do sul do país (CASTIGLIONI et al., 2007; CASTIGLIONI; BOND-BUCKUP, 2008a, b, 2009). Além disso, os trabalhos de Torres (2012) e Bastos-Pereira (2014) elucidam o ciclo de vida e a dinâmica populacional de duas espécies de *Hyaella* encontradas em Minas Gerais. Rodrigues (2011) estudou a distribuição geográfica do gênero no Rio Grande do Sul, e Rodrigues (2016), dentre outras coisas, estudou a biogeografia do gênero na América do Sul. Para a região sul do Brasil, as pesquisas foram sobre reprodução e dinâmica populacional de *H. bonariensis* Bond-Buckup, Araújo e Santos, 2008 (Castiglioni et al. 2016, 2018) e a biologia reprodutiva de *H. georginae* Streck e Castiglioni, 2017 e *H. gauchensis* Streck e Castiglioni, 2017 (Ozga e Castiglioni 2017).

Até o momento, a única pesquisa sobre o gênero para o Parque Estadual da Serra do Mar – Núcleo Santa Virgínia (PESM – NSV) é um trabalho de conclusão de curso com a descrição de uma espécie do gênero (PENONI, 2016). O mesmo ainda não foi publicado.

Esta dissertação apresenta, em forma de artigo científico, pesquisa realizada com o gênero *Hyaella* encontrado no PESP – NSV. O objetivo do trabalho é analisar os fatores do ambiente físico que moldam a coexistência de diferentes fases de vida e de dois diferentes morfotipos do gênero *Hyaella* através da divisão de nicho e as estratégias reprodutivas dos morfotipos, com base em dados de fecundidade.

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*For Biota Neotropica*

## **Ecological traits of two *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) morphotypes from Southeastern Brazil**

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### **Abstract:**

*According to the niche theory, coexistence of cryptic species in nature depends either on niche division or on intrinsic mechanisms that regulate a species. Hence, niche division would allow the physical coexistence of similar species. Here we ask if two different *Hyalella* morphotypes, as well as their life stage categories, coexist based on niche differentiation, and if they present different reproductive strategies regarding fecundity. This research took place at Parque Estadual da Serra do Mar - Núcleo Santa Virgínia, a state park from Southeast Brazil. Morphotypes differed on antennas to head length ratios, being morphotype 1 (M1) the smallest and morphotype 2 (M2) the bigger one. Multiple regression analysis were used to identify the physical habitat variables that most relate to each morphotype and life stage category abundance. M1 organisms were negatively associated to the occurrence of boulders along the transects. Females and ovigerous females were closely related to this variable; males were positively affected by mean substrate size; and juveniles did not respond to any physical habitat variable. M2 organisms, on the other hand, were negatively affected by canopy coverage. Females and ovigerous females were dependent only upon this variable; males responded negatively to canopy coverage and positively to wetted width; and juveniles displayed preference to areas with less canopy coverage and more availability of brush and small wood debris. The mean number of eggs in the ovigerous females' marsupium differed between morphotypes, with M2 females bearing more eggs. Mean egg volume and mean volume of eggs in the marsupium also differed between morphotypes, with M2 females carrying more voluminous eggs. In summary, the different factors which explained abundance produced different occupation and, the hypothesis that the two morphotypes and the different life stages occupy different niches was endorsed by our results.*

**Keywords:** Physical habitat – Niche division – Morphology – Fecundity

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### **Introduction**

The occurrence of sympatric cryptic species presents a challenge to the typical understanding of species coexistence (Leibold & McPeck 2006). They share ecological and morphological traits, *i.e.* cryptic species exhibit very high phenotypic similarities (Wellborn & Cothran 2007). Some theories and mechanisms could explain their coexistence. For example, non-equilibrium dynamics could enable two very similar species to persist together for long periods of time (McPeck & Gomulkiewicz 2005; Wellborn & Cothran 2007), while the niche division theory proposes that with enough trade-offs, even species with similar responses to environmental factors can coexist (Leibold & McPeck 2006). Indeed, the niche theory is well known and enfolded the basis of community ecology (Chase & Leibold 2003).

For two similar phenotypes to coexist, they need to present enough differences in relevant ecological traits, those that compose a species' niche (Chase & Leibold 2003). Nevertheless, ecological variations do not guarantee a stable coexistence; it depends on a combination of equalizing and stabilizing mechanisms (Chase & Leibold 2003; Leibold & McPeck 2006). Interspecific competition in this context can lead to body size partitioning: in order to reduce competition, species become more and more different in their mean body size (Harikrishnan & Vasudevan 2018).

The amphipod genus *Hyalella* Smith, 1874 occurs in a variety of freshwater environments, such as rivers, temporary ponds, the travertine in caves, springs and wetlands (Grosso & Peralta 1999; Bastos-Pereira & Bueno 2012; Rodrigues et al. 2012, 2014). Few studies investigate the ecological roles and interactions of the genus, specially the South America species (Castiglioni et al. 2007, 2016, 2018; Castiglioni & Bond-Buckup 2007, 2008a, b, 2009; Torres et al. 2015; Bastos-Pereira & Bueno 2016a, b; Ozga & Castiglioni 2017). For some North American species, their relation to the environment and many trade-offs that occur with sympatric species are well known (Wellborn 1994, 2002; Wellborn & Cothran 2004, 2007). Instead, not much is known about coexistence of different species and niche division in tropical *Hyalella*. Except for Castiglioni & Bond-Buckup (2007, 2008a, b, 2009) and Castiglioni et al. (2007) research, all other ecological studies investigate single species. Other sympatries are known: *H. curvispina* Shoemaker, 2941 and *H. bonariensis* Bond-Buckup, Araújo & Santos, 2008, in Bom Jesus, Rio Grande do Sul, Brazil; and *H. bonariensis* and *H. pleoacuta* González, Bond-Buckup & Araújo, 2006 in Caçapava do Sul, Rio Grande do Sul, Brazil (unpublished data), but it needs further investigation.

Here we present a primary study of the ecology of two different *Hyalella* morphotypes encountered at Southeastern Brazil. The objectives were to answer two questions: a) is the coexistence of different *Hyalella* morphotypes and life stage categories niche-based in terms of habitat use?; and b) do the different morphotypes present different reproductive strategies regarding fecundity?

## Material and Methods

### Study area

Collections were held at The Parque Estadual da Serra do Mar - Núcleo Santa Virgínia (PESM - NSV), São Luiz do Paraitinga municipality, São Paulo state, Brazil (Figure 1). The site is located at Paraíba do Sul river basin, and the tributary Paraíba river is the main water course encompassing the area (Villani 1998). The regional altitude varies from 860 m to 1500 m. PESM – NSV vegetation is mostly composed by the Atlantic rainforest, bearing important endemic species (Villani 1998).

### **Sampling procedures**

#### **Physical habitat data**

Two different wadable streams were analyzed in the “*Poço do Pito*” trail. For the first one, “*Pau de Bala*” Stream (23S 19°24,5”; 45W 07°53,5”), ten transects (A to J, downstream to upstream) were evaluated and sampled. This portion of the stream is characterized by a closed, preserved riparian vegetation, mostly high flow water (riffles and rapids), mean wet width of 2.723 m, and water depth not more than 37 cm (mean depth: 13.48 cm) during the collection period. For the second one, “Fen 1” stream (23S 19°36,5”; 45W 08°01,1”), two transects (K and L) were evaluated and sampled. This is a smaller water body, that branches from the “*Pau de Bala*” stream and breaks in a fall a few meters after transect K. This portion of the stream is characterized by a narrower (mean wet width: 0.765 m), deeper water column (mean depth: 28.8 cm), slow moving water and open riparian vegetation.

Physical habitat data were obtained for each transect, using the Channel/Riparian Cross Section form based on Kaufmann & Robison (1998). Each transect was 15 m apart from the previous one. For each transect, a 10 m line (5 m upstream and 5 m downstream) on both margins was used to observe habitat complexity and cover and riparian vegetation structure. On the transect line, channel substrate size and type (based on the protocol), vegetation cover through densiometer, and water depth were collected five times using a cane with scale: on both left and right margins, on the middle, and between the middle and the margins. Any other observations were recorded. All these data was than processed and combined on 19 variables: (1) mean depth; (2) mean substrate size; (3) mean substrate immersion (standardized by the protocol); (4 – 12) abundance (classified as: 0 – absent; 1 – sparse; 2 – moderate; 3 – heavy; 4 – very heavy) of filamentous algae, aquatic macrophytes, large wood debris, brush and small wood debris, living trees and roots, leaf litter, overhanging vegetation, undercut banks, boulders; (13) sum of 4 – 12; (14) mean bank angle; (15) mean undercut bank distance; (16) wettet width; (17) bankfull width; (18) bankfull high; and (19) canopy cover.

For characterizing the two streams, chemical data was recorded before organism collection to prevent any disturbances. On the first transect of both streams (A and K) we measured the pH, water conductivity; and dissolved oxygen using specific digital portable meters. The water discharge for both streams was estimated using three depths and three counts of time of a floating object traveling a known distance on a known area. Table 1 presents the results.

## Crustacean collection

*Hyalella* organisms were collected in May 2018. The following protocol was used to collect and preserve the crustaceans: two people collecting simultaneously using hand nets, one minute collection along the right margin of the stream 5 meters up and downstream from the transect line. On field triage was performed for one minute: the material captured at the hand nets was transferred to a white tray and all *Hyalella* found were preserved on alcohol 70%. Ovigerous females (females carrying eggs or juveniles on the incubation pouch) were individualized. This process was repeated on the left margin, and for each transect.

## Organisms identification

For organism identification, they were all photographed using the Carl Zeiss Stemi 508 stereomicroscope with the AxioCam ERc5s camera attached. Head length (HL) (measured from the division between torax and head to the antenna 1 attachment spot), antenna 1 total length (A1) (from the basis of the peduncle to the tip of the last article), antenna 2 total length (A2), peduncle length (PA2) and ramus length (RA2) were measured with the help of Carl Zeiss AxioVision program SE64 Rel 4.8.3 software. They were all identified in one of the following life stage categories: males, females, ovigerous females and juveniles.

Visual observation indicated two different morphotypes, one with smaller antennas, and another with rather longer, more developed ones (Figure 1). The ratios PA2/A1 and A2/HL were used to separate organisms: morphotype 1 (M1): animals with  $PA2/A1 < 1.00$  and  $A2/HL < 7.29$ ; and morphotype 2 (M2):  $PA2/A1 \geq 1.00$  and  $A2/HL \geq 7.29$ . Those organisms that did not meet the expectations for both ratios (86 in total) to be classified as M1 or M2 were then observed regarding the ratio PA2/BA2: organisms with  $PA2/BA2 < 1.62$  were classified as M1 and  $PA2/BA2 \geq 1.62$  as M2.

Organisms with head length smaller than the smallest ovigerous female were considered juveniles (0.390 mm for M1 and 0.467 mm for M2).

## Fecundity observations

To account for reproductive differences between the morphotypes, we counted the number of eggs in the marsupium of ovigerous females and approximated the mean volume of eggs per female. Eggs were removed from the marsupium with the aid of fine insulin needles, and counted and measured under the stereomicroscope with an attached microscale. Only females with eggs on embryonic stage 1 (little or no cleavage, based on Hynes 1955 and Subida et al. 2005) were used.

In order to obtain the mean volume of an egg in the marsupium of each female and the mean egg volume per female, five random eggs were selected per female. The length ( $l$ ) - the longest axis - and width ( $w$ ) - the perpendicular axis in relation to length - were measured. Volume was calculated using the following equation (Beyer 1987 as cited by Wilhelm & Schindler 2000):

$$V = \frac{4}{3} \pi r_1 r_2 r_3$$

eqn 1

where  $V$  = volume ( $\text{mm}^3$ );  $r_1$  = length/2 (mm);  $r_2$  and  $r_3$  = width/2 (mm). Depth and width were assumed to be equivalent, hence depth wasn't measured.

### Data Analysis

Morphological differences between the morphotypes were evaluated using a Principal Component Analyses (PCA). A discriminant analyses was performed to explore the results of this PCA, testing which factors separate the groups.

A second PCA was performed to evaluate if the abundance of the different morphotypes is related to different physical habitat components, and if special segregation is evident. The most important variables that differentiate the sampled transects were used as explanatory variables of the abundance for each category (M1; M2; males, total females, ovigerous females, juveniles of each morphotype) in a multiple regression analysis. The statistical analysis were performed using the Statistica 13.2 software.

The fecundity (number of eggs) was compared between morphotypes using a test  $t$  ( $\alpha = 0.05$ ). Different mean egg volume and mean volume of eggs per female between morphotypes were also compared using test  $t$  ( $\alpha = 0.05$ ). By means of a regression analysis we assessed the number of eggs in relation to ovigerous females head length. These analysis were performed using Excel 2016 Data Analysis ToolPack.

### Results

A total of 759 organisms were found (M1 = 356; M2 = 403), with predominance of females for both morphotypes (Table 2).

PCA analysis indicated that morphotypes M1 e M2 are morphologically distinct based on antennas and head lengths (Table 3, Figure 2). Such analyses also pointed out that M2 had a greater morphological variability. Factor 1, which combined all three ratios (PA2/A1, A2/HL and PA2/RA2) explained 86.29% of variations. The discriminant analysis test confirmed the differences found on the PCA ( $p < 0.0000$ ) (Table 4).

The sampled transects presented diferent physical-habitat conditions. However, whilst M1 organisms were more abundant in spaces that combine lower mean depth, more boulders and more canopy coverage, M2 organisms tended to inhabit spaces with greater depth, less boulders and less canopy coverage (Table 5; Figures 3 and 4).

The presence of algae positively influences the presence of *Hyaella* when morphotypes were analyzed together ( $p = 0.001785$ ) (Table 6, Figure 5). Eventhough the streams had only transecs with absent or sparce algae (classified as either 0 or 1, in a scale of 0 to 4), the amphipods were preferably found associated with such organisms.

The main habitat component associated to M1 was undercut banks, which was negatively related to abundance. Multiple regression best fitted models for total abundance ( $N = 356$ ;  $p = 0.029994$ ), females ( $N = 200$ ;  $p = 0.008172$ ) and ovigerous females ( $N = 59$ ;  $p = 0.000446$ ) all resulted on this variable as the only one associated to the categories (Tables 7, 9 – 10, Figures females ( $N = 59$ ;  $p = 0.000446$ ) all resulted on this variable as the only one associated to the categories (Tables 10, 12 – 13, Figures 6 and 8). Differently, males best fitted regression models resulted in mean substrate size positive dependence ( $N = 92$ ;  $p = 0.002425$ ) (Table 8, Figure 7). Juveniles ( $N = 64$ ) did not respond to any habitat component.

Morphotype 2 main habitat component dependence relied on canopy coverage. All life stage categories were negatively affected by this variable (Tables 11 – 15, Figures 9 – 13). When analyzing categories altogether ( $N = 403$ ), both bankfull height ( $p = 0.014718$ ) and canopy coverage ( $p = 0.000004$ ) negatively affected abundance (Figure 9). For males ( $N = 146$ ), wetted width ( $p = 0.000292$ ) and canopy coverage ( $p = 0.000035$ ) composed the physical habitat variables determining abundance (Figure 10), with the variables acting in different directions. Total females ( $N = 180$ ) and ovigerous females ( $N = 65$ ) (Figure 11) presented dependence only upon canopy coverage ( $p = 0.000000$  and  $p = 0.000001$ , respectively). Juveniles ( $N = 77$ ), in addition to canopy coverage ( $p = 0.000232$ ), were also dependent on the disponibility of brush and small wood debris ( $p = 0.001173$ ), meaning that with lower canopy coverage and more brush and small wood debris on the water, greater M2 juvenile abundance (Figure 12).

Regression analysis of fecundity against size indicated a positive relation between head length and number of eggs, meaning that bigger females have a bigger brood (Figures 13 and 14). However, fecundity was statistically different between morphotypes. Egg production ranged from two to 31 ( $n = 54$ ) in M1 and from one to 33 ( $n = 64$ ) in M2. There was significant difference between the mean number of eggs produced by M1 ( $9.35 \pm 4.25$ ) and M2 ( $15.75 \pm 6.97$ ) ( $t = -6.17$ ;  $P < 0.05$ ). There were also significant differences of mean egg volume between morphotypes ( $t = 1.66$ ;  $P < 0.05$ ), with mean egg volume of M1 =  $0.024 \pm 0.008 \text{ mm}^3$  and mean egg volume of M2 =  $0.026 \pm 0.009 \text{ mm}^3$ . Thereafter, we also found significant differences between mean volume of eggs per female between morphotypes (M1:  $0.22 \pm 0.11 \text{ mm}^3$ ; M2:  $0.44 \pm 0.23 \text{ mm}^3$ ).

## Discussion

This research presents the first insights on the ecology of *Hyalella* morphotypes at Parque Estadual da Serra do Mar – Núcleo Santa Virgínia. We used for the first time protocols of physical habitat analysis to explore habitat use on this amphipod genus. Here we observed two distinct phenotypes responsive to different environmental variables as well as different life stage categories associated to different physical habitat components. Our research also included reproductive analysis, indicating that fecundity vary among morphotypes.

First, we found that *Hyalella* morphotypes 1 and 2 are morphologically distinct based on antenna and head length ratios. Morphotype 1 organisms were in general small-bodied with both antennae short, while morphotype 2 organisms were larger, more robust, and with antenna 2 much longer than the head and antenna 1. Natural freshwater communities can bear hidden diversity in form of cryptic species, and recent genetic studies are useful to reveal the concealed diversity (Wellborn & Cothran 2007). *Hyalella* assemblages have been reported on several glacial lakes in the northern USA (Wellborn & Cothran 2004, 2007), and in southern Brazilian ponds (Castiglioni et al., 2007; Castiglioni & Bond-Buckup 2007, 2008a, b, 2009); other example of hidden *Hyalella* diversity is proposed by González & Watling (2003), who indicated a richness of about 100 undescribed species for the Titicaca lake between Peru and Bolivia, .

For some *Hyalella* assemblages, identifying organisms can be easy, based on evident, diagnostic morphological characteristics. For example, *H. castroi* González, Bond-Buckup & Araújo, 2006 and *H. pleoacuta*, found on the same trout ponds in southern Brazil, can be straight separated by the naked eye, because while the first has a smooth body, the second one presents dorsal spines, known as flanges, that are easily identified (González et al. 2006). Nevertheless, genetic analysis grouped them as a single clade (Rodrigues 2016). The two morphotypes herein explored were harder to identify. Eventhought the larger M2 organisms had distinct, more developed antennae, the smaller ones could be easily mistaken as M1. Because of that, more detailed observations were needed. All individuals had to be meticulously observed under the microscope, and antennae and the head had to be measured. These measurements and the ratios encountered were then used to classify the organisms as either morphotypes. In addition to antenna length ratios, the morphotypes can be separated by gantopod 2 propodus' morphology (personal observation).

When exploring both morphotypes combined, we found that the presence of algae on the water was a good evidence of the presence of *Hyalella*. Eventhough both streams had only none or little algae presence, this was an expected result considering that *Hyalella* is a genus of detritivores omnivours (Bousfield 1996). Filamentous algae provide food and shelter, and can increase the structural complexity. According to Czarnecka et al. (2010), the substrates' physical structure influences gammarids habitat selection, but organisms can show some plasticity depending on body size.

The small bodied, small antenna organisms herein studied tend to inhabit shallower, fast flowing waters, usually associated to closed riparian vegetation. They were also negatively associated to undercut banks. These variables are related to habitat structure complexity and environmental stability. Because they are smaller, their competitive ability is assumably minor (Wellborn 2002), and occupying a more structured, complex portion of a stream facilitate food capture and protection availability. On the other hand, M2 was more frequently found on deeper, stagnant water, and associated to more open areas. The foraging requirement increases with body mass (Wellborn 1994), and bigger organisms, which can easily move around their habitat area, do not need constant proximity with their food (Czarnecka et al. 2010), *i.e.* they are able to inhabit poorer

portions of the environment. The dissociation of M2 with canopy coverage is recurrent to all life stage categories, reinforcing that this morphotype bears more unprotected, less structured conditions.

All life stage categories of both morphotypes were associated to different environmental variables, except for M1 juveniles, which did not respond to any physical habitat component. These divergences show a clear niche division based on habitat occupation between morphotypes and between life stage categories within a morphotype. Three sympatric cryptic *Hyalella* species from Oklahoma, USA exhibited a spatial segregation: the bigger species was vertically separated from the smallest ones, occupying the shallower, most marginal area, whilst the two smallests were horizontally separated, inhabiting different depths in the water column (Wellborn & Cothran 2007). Similarly, south Brazil sympatric *Hyalella* species, *H. castroi* and *H. pleoacuta*, differ on macrophyte preferences, with species exploring different resource and refuge sources (González et al. 2006). Czarnecka et al. (2010) demonstrated that adults and juveniles of *Pontogammarus robustoides* Sars, 1894 prefer different substrates, thus, selecting portions of the habitat that provide more adequate food and protection based on body size.

Males depended upon mean substrate size (M1) and wetted width (M2). These variables are directly associated to space, which, accordingly, relate to local dispersal ability, meaning that males more easily travel from one transect to another. Although the physical properties of a substrate help gammarids determine habitat quality, protection opportunities are more important to adults than food availability (Czarnecka et al. 2010). More open, less complex spaces are then preferred by them (Czarnecka et al. 2010). They provide protection and, because gammarids use chemical signals to perceive quality (De Lange et al. 2005), the openness of the environment can also ease the access to food.

Morphotype 2 juveniles, besides canopy coverage were also associated to brush and small wood debris on the water. This variable represents protection and food availability, and, again, provides more complexity. Juveniles can adhere to small surface rugosities, which increases the habitat quality (Czarnecka et al. 2010).

Ovigerous and non-ovigerous females shared the same dependence within a morphotype, indicating that their feeding, protection and general fitness needs are similar when carrying eggs or not. Differences in fecundity (number of eggs) were directly related to body size. M2 fecundity was higher than M1's, and bigger females within a morphotype bore more eggs. With our data, we cannot foresee differences on incubation time and viability of eggs such the ones encountered by Castiglioni & Bond-Buckup (2007), but our results suggest that, even though we found statistical differences regarding fecundity, the morphotypes reproductive strategies seem to be similar. More details on this aspect are needed to discuss further differences between the behavioral ecology of these morphotypes.

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## Appendices

### Tables

**Table 1. Parque Estadual da Serra do Mar – Núcleo Santa Virgínia streams “Pau de Bala” and “Fen 1” low flow dischard and chemical characterization.**

Stream	pH	Conductivity ( $\mu\text{S/cm}$ )	Dissolved oxygen (mg/L)	Water dischard (m <sup>3</sup> /s)
Pau de Bala	6.75	16	7.3	68.7
Fen 1	7.29	17	7.2	1

**Table 2. Correlation matrix (variables against number of individuals of *Hyalella* sp.). Variables correspond to physical habitat observations. Marked correlations are significant at  $p < 0.05000$ . Legend: Xdepth: mean depth; Xsub: mean substrate size; Xsubimmer: mean substrate immersion; XFC\_alg: filamentous algae; XFC\_aqm: aquatic macrophytes; XFC\_lwd: large wood debris; XFC\_irs: brush and small wood debris; XFC\_trr: living trees and roots; XFC\_llt: leaf litter; XFC\_ohv: overhanging vegetation; XFC\_odb: undercut banks; XFC\_boul: boulders; SUM\_FC: sum of all “XFC\_” variables; Xbankang: mean bank angle; Xundbdis: mean undercut bank distance; WETwidth: wettet width; BANKwidth: bankfull width; BANKhei: bankfull height; and CANcover: canopy cover.**

Variables	<i>Hyalella</i> (N)
Xdepth	0.515731771
Xsub	0.098164775
Xsubimmer	0.297356315
XFC_alg	0.799926374
XFC_aqm	0.685504273
XFC_lwd	0.187474862
XFC_irs	-0.154703142
XFC_trr	0.418864004
XFC_llt	-0.457133132
XFC_ohv	0.073687268
XFC_odb	-0.058524626
XFC_boul	-0.704194518
SUM_FC	-0.237265664
Xbankang	-0.518168645
Xundbdis	0.514902984
WETwidth	0.395928431
BANKwidth	-0.201303686
BANKhei	-0.42738944
CANcover	-0.785677969

**Table 3. Correlation matrix (variables against number of individuals of *Hyalella* sp. morphotype 1 life stages). Variables correspond to physical habitat observations. Marked correlations are significant at  $p < 0.05000$ . Legend: total M1: sum of males, females and juveniles of *Hyalella* sp. morphotype 1; M1: morphotype 1; Fem: females; Juv: juveniles; Ov: ovigerous; Xdepth: mean depth; Xsub: mean substrate size; Xsubimmer: mean substrate immersion; XFC\_alg: filamentous algae; XFC\_aqm: aquatic macrophytes; XFC\_lwd: large wood debris; XFC\_irs: brush and small wood debris; XFC\_trr: living trees and roots; XFC\_llt: leaf litter; XFC\_ohv: overhanging vegetation; XFC\_odb: undercut banks; XFC\_boul: boulders; SUM\_FC: sum of all “XFC\_” variables; Xbankang: mean bank angle; Xundbdis: mean undercut bank distance; WETwidth: wettet width; BANKwidth: bankfull width; BANKhei: bankfull height; and CANcover: canopy cover.**

Variables	total M1	Males M1	Fem. M1	Juv. M1	Ov.Fem. M1
Xdepth	-0.53719296	-0.41423564	-0.46332482	-0.49090378	-0.27969331
Xsub	0.522666101	0.786231292	0.342682834	0.249729239	0.04074867
Xsubsummer	-0.29976358	-0.21993035	-0.34234246	-0.10870756	-0.4225811
XFC_alg	0.154719442	0.192879187	0.078956767	0.147979087	-0.32166449
XFC_aqm	-0.13058692	-0.28068045	-0.04308711	-0.0717804	-0.13549991
XFC_lwd	-0.56087537	-0.46315109	-0.53514693	-0.32476831	-0.45897939
XFC_brs	0.11815542	-0.08183171	0.244260036	0.034879005	0.191537802
XFC_trr	-0.004503	-6.2324E-17	-0.02872474	0.0358902	0.11086356
XFC_llt	0.260880902	0.187835721	0.244407287	0.212733341	0.124830343
XFC_ohv	0.401722707	0.377964473	0.326368897	0.302061047	0.000000000
XFC_udb	-0.6243603	-0.42586571	-0.72079503	-0.20944165	-0.851109
XFC_boul	0.170436169	-0.09207131	0.235563689	0.239988586	0.108788549
SUM_FC	0.073175806	-0.13683565	0.113780181	0.204131378	-0.17515406
Xbankang	-0.21488767	-0.28064849	-0.26833917	0.144054386	-0.23299043
Xundbdis	-0.22692876	0.107563755	-0.39309948	-0.08737827	-0.46482036
WETwidth	0.071089738	0.514085071	-0.14062907	-0.030276	-0.29622957
BANKwidth	0.015926614	0.0201059	0.051467063	-0.10275405	0.12578603
BANKhei	0.238277937	0.143583732	0.358808814	-0.03405704	0.382974164
CANcover	0.362906478	0.173774295	0.314235227	0.435363789	0.079085129

**Table 4. Correlation matrix (variables against number of individuals of *Hyalella* sp. morphotype 2 life stages). Variables correspond to physical habitat observations. Marked correlations are significant at  $p < 0.05000$ . Legend: total M2: sum of males, females and juveniles of *Hyalella* sp. morphotype 2; M2: morphotype 2; Fem: females; Juv: juveniles; Ov: ovigerous; Xdepth: mean depth; Xsub: mean substrate size; Xsubsummer: mean substrate immersion; XFC\_alg: filamentous algae; XFC\_aqm: aquatic macrophytes; XFC\_lwd: large wood debris; XFC\_brs: brush and small wood debris; XFC\_trr: living trees and roots; XFC\_llt: leaf litter; XFC\_ohv: overhanging vegetation; XFC\_udb: undercut banks; XFC\_boul: boulders; SUM\_FC: sum of all “XFC\_” variables; Xbankang: mean bank angle; Xundbdis: mean undercut bank distance; WETwidth: wetted width; BANKwidth: bankfull width; BANKhei: bankfull height; and CANcover: canopy cover.**

Variables	total M2	Males M2	Fem. M2	Juv. M2	Ov.Fem. M2
Xdepth	0.744691026	0.629959034	0.806579729	0.548089986	0.823911777
Xsub	-0.13131348	0.138444526	-0.25691722	-0.40742635	-0.45902376
Xsubsummer	0.425012778	0.484975678	0.35880592	0.381595028	0.277512222
XFC_alg	0.723374005	0.783823937	0.659460597	0.494517714	0.546268045
XFC_aqm	0.734897063	0.550817714	0.792274246	0.757907114	0.855810307
XFC_lwd	0.430453817	0.275032588	0.502896354	0.350451623	0.59849165
XFC_brs	-0.20460107	-0.31738943	-0.13736236	-0.08927919	0.027839268
XFC_trr	0.416146529	0.297344076	0.390557727	0.727284604	0.397468385
XFC_llt	-0.56601258	-0.60861215	-0.55544998	-0.28251147	-0.47721092
XFC_ohv	-0.10267068	0.123074916	-0.21287452	-0.25772682	-0.32547843
XFC_udb	0.21494578	0.254873473	0.201433215	0.028592224	0.193916498
XFC_boul	-0.77079048	-0.78841761	-0.74991414	-0.48873128	-0.61892641
SUM_FC	-0.26658616	-0.34457562	-0.24782529	-0.03421207	-0.10037708
Xbankang	-0.41847713	-0.39381074	-0.46248556	-0.14235399	-0.41762722

Xundbdis	0.608300787	0.748069072	0.513051775	0.318569243	0.328360987
WETwidth	0.36043706	0.642376166	0.209874809	-0.06056073	-0.04464425
BANKwidth	-0.20601124	-0.24896339	-0.15279224	-0.17043139	-0.17493079
BANKhei	-0.52672515	-0.57726485	-0.46879669	-0.43085697	-0.34695655
CANcover	-0.93546291	-0.79376728	-0.96888049	-0.81732688	-0.96224827

**Table 5. Total number of *Hyalella* sp. found at Parque Estadual da Serra do Mar – Núcleo Santa Virgínia.**

Category	N
Morphotype 1 (M1)	356
Male (M1)	92
Female (ovigerous + non-ovigerous) (M1)	200
Ovigerous female (M1)	59
Juvenile (M1)	64
Morphotype 2 (M2)	403
Male (M2)	146
Female (ovigerous + non-ovigerous) (M2)	180
Ovigerous female (M2)	65
Juvenile (M2)	77

**Table 6. Principal Componente Analysis summary table. *Hyalella* sp. morphotypes 1 and 2 are statistically separated by the ratios “Antenna 2 peduncle length (PA2)/Antenna 1 total length (A1)”; “Antenna 2 total length (A2)/head length (HL)”; and “PA2/Antenna 2 ramus length (RA2)”.**

Variable	Factor coordinates of the variables, based on correlations		
	Factor 1	Factor 2	Factor 3
PA2/A1	-0.936532	0.191196	0.293858
A2/HL	-0.914455	-0.404637	-0.006367
PA2/RA2	-0.935657	0.204093	-0.287910

**Table 7. Discriminant function analysis summary for *Hyalella* sp. morphotypes 1 and 2. Ratios “Antenna 2 peduncle length (PA2)/Antenna 1 total length (A1)”; “Antenna 2 total length (A2)/head length (HL)”; and “PA2/Antenna 2 ramus length (RA2)” well explain the morphotypes differences.**

N = 741	Number of vars in model: 3; Grouping: Morphotype (2 grps); Lambda: 0.23448; approx. F (3,737) = 802.04; p < 0.0000				Wilks'	
	Wilks'	Partial	F-remove	p-value	Toler.	1-Toler.
PA2/A1	0.236264	0.992452	5.6049	0.018167	0.648802	0.351198
A2/HL	0.270342	0.867351	112.7134	0.000000	0.807289	0.192712
PA2/BA2	0.308640	0.759724	233.0897	0.000000	0.712404	0.287596

**Table 8. Principal Componente Analysis summary table. *Hyalella* sp. morphotypes 1 and 2 statistically occupy different environmental niches. Factors 1 and 2 combined explain 50.82% of variations. Legend: Xdepth: mean depth; Xsub: mean substrate size; Xsubimmer: mean substrate immersion; XFC\_alg: filamentous algae; XFC\_aqm: aquatic macrophytes; XFC\_lwd: large wood debris; XFC\_brs: brush and small wood debris; XFC\_trr: living trees and roots; XFC\_llt: leaf litter; XFC\_ohv: overhanging vegetation; XFC\_udb: undercut banks; XFC\_boul: boulders; SUM\_FC: sum of all “XFC\_” variables; Xbankang: mean bank angle; Xundbdis: mean undercut bank distance; WETwidth: wetted width; BANKwidth: bankfull width; BANKhei: bankfull height; and CANcover: canopy cover.**

Variable	Factor - variable correlations (factor loadings), based on correlations			
	Factor 1	Factor 2	Factor 3	Factor 4

Xdepth	0.796185	0.364764	-0.266239	0.149788
Xsub	-0.016303	-0.862773	-0.077575	-0.152443
Xsubsimmer	0.565196	0.046287	-0.287808	-0.389582
XFC_alg	0.800932	-0.021620	-0.258697	-0.129450
XFC_aqm	0.660148	0.586544	-0.147843	0.149752
XFC_lwd	0.399739	0.691895	0.299028	-0.171795
XFC_brs	-0.329298	0.489414	-0.687636	0.035788
XFC_trr	0.178579	0.301755	0.529151	-0.095434
XFC_ilt	-0.696419	0.169821	0.390899	-0.246570
XFC_ohv	0.051919	-0.598571	-0.660914	-0.259681
XFC_udb	0.389309	0.334071	0.040591	-0.639108
XFC_boul	-0.811867	0.275363	-0.294410	-0.242145
SUM_FC	-0.354936	0.630209	-0.377519	-0.480326
Xbankang	-0.392508	0.059889	0.452706	-0.618124
Xundbdis	0.750292	-0.325131	0.020697	-0.250517
WETwidth	0.532939	-0.715287	-0.019779	-0.285454
BANKwidth	-0.190790	-0.005704	0.217796	0.408283
BANKhei	-0.602082	0.134569	-0.644667	0.117828
CANcover	-0.840357	-0.387072	0.021957	-0.202986

**Table 9. Best fitted multiple regression analysis summary for total number of individuals (*Hyalella* sp. morphotype 1 combined with morphotype 2).**

<b>R = 0.79992637; R<sup>2</sup> = 0.63988220; Adjusted R<sup>2</sup> = 0.60387042;</b>						
<b>F (1,10) = 17.769; p &lt; 0.00178; Std. Error of estimate: 23.130</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			47.00000	7.71002	6.095962	0.000116
Algae	0.799926	0.189768	65.00000	15.42004	4.215293	0.001785

**Table 10. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 1 abundance (life stage categories “males”, “females” and “juveniles” combined). Legend: XFC\_udb: average undercut bank.**

<b>R = 0.62436030; R<sup>2</sup> = 0.38982579; Adjusted R<sup>2</sup> = 0.32880837;</b>						
<b>F (1,10) = 6.3888; p &lt; 0.02999; Std. Error of estimate: 13.305</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			44.73080	7.14570	6.259820	0.000094
XFC_udb	-0.624360	0.247017	-11.42310	4.51934	-2.527600	0.029994

**Table 11. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 1 male abundance. Legend: Xsub: average substrate size.**

<b>R = 0.78623129; R<sup>2</sup> = 0.61815964; Adjusted R<sup>2</sup> = 0.57997561;</b>						
<b>F (1,10) = 16.189; p &lt; 0.00242; Std. Error of estimate: 3.3770</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			0.51320	2.02763	0.253120	0.805307
Xsub	0.786231	0.195407	0.05360	0.01332	4.023550	0.002425

**Table 12. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 1 female abundance. Legend: XFC\_ubd: average undercut bank.**

<b>R = 0.72079503; R<sup>2</sup> = 0.51954547; Adjusted R<sup>2</sup> = 0.47150002; F (1,10) = 10.814; p &lt; 0.00817; Std. Error of estimate: 7.4030</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			27.69230	3.97603	6.964820	0.000039
XFC_udb	-0.720795	0.219193	-8.26920	2.51466	-3.288410	0.008172

**Tables 13. Best fitted multiple regression analysis summary for *Hyalella* morphotype 1 ovigerous female abundance. Legend: XFC\_udb: average undercut bank.**

<b>R = 0.85110900; R<sup>2</sup> = 0.72438653; Adjusted R<sup>2</sup> = 0.69682519; F (1,10) = 26.283; p &lt; 0.00045; Std. Error of estimate: 1.6344</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			8.71150	0.87780	9.924340	0.000002
XFC_udb	-0.851109	0.166016	-2.84620	0.55517	-5.126670	0.000446

**Table 14. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 2 abundance (life stage categories “males”, “females” and “juveniles” combined). Legend: BANKhei: bankfull height; CANcover: canopy cover.**

<b>R = 0.96837547; R<sup>2</sup> = 0.93775105; Adjusted R<sup>2</sup> = 0.92391794; F (2,9) = 67.790 p &lt; 0.00000; Std. Error of estimate: 10.251</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(9)</b>	<b>p-value</b>
Intercept			152.82700	10.67132	14.321320	0.000000
BANKhei	0.263158	0.087431	-13.57400	4.50972	-3.009890	0.014718
CANcover	0.854271	0.087431	119.32000	12.21195	-9.770790	0.000004

**Table 15. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 2 male abundance. Legend: WETwidth: wetted width; CANcover: canopy cover.**

<b>R = 0.95912157; R<sup>2</sup> = 0.91991419; Adjusted R<sup>2</sup> = 0.90211734; F (2,9) = 51.690; p &lt; 0.00001; Std. Error of estimate: 4.6936</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(9)</b>	<b>p-value</b>
Intercept			42.44000	4.80063	8.840470	0.000010
WETwidth	0.543535	0.095236	0.46700	0.08176	5.707270	0.000292
CANcover	0.719053	0.095236	-40.54000	5.36938	-7.550260	0.000035

**Table 16. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 2 female abundance. Legend: CANcover: canopy cover.**

<b>R = 0.96888049; R<sup>2</sup> = 0.93872940; Adjusted R<sup>2</sup> = 0.93260234; F (1,10) = 153.21 p &lt; 0.00000; Std. Error of estimate: 5.1039</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>
Intercept			74.54700	5.04423	14.778700	0.000000
CANcover	0.968880	0.078276	-71.58600	5.78343	12.377800	0.000000

**Table 17. Best fitted multiple regression analysis summary for *Hyalella* sp. morphotype 2 ovigerous female abundance. Legend: CANcover: canopy cover.**

<b>R = 0.96224827; R<sup>2</sup> = 0.92592172; Adjusted R<sup>2</sup> = 0.91851390; F (1,10) = 124.99; p &lt; 0.00000; Std. Error of estimate: 2.9148</b>						
<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(10)</b>	<b>p-value</b>



Intercept			36.21900	2.88071	12.572800	0.000000
CANcover	0.962248	0.086069	-36.92600	3.30287	11.180000	0.000001

**Table 18. Best fitted multiple regression analysis summary for *Hyaella* sp. morphotype 2 juvenile abundance. Legend: XFC\_trr: average living trees and roots; CANcover: canopy cover.**

**R = 0.95023054; R<sup>2</sup> = 0.90293808; Adjusted R<sup>2</sup> = 0.88136877;  
F (2,9) = 41.862; p < 0.00003; Std. Error of estimate: 1.6450**

<b>N=12</b>	<b>b*</b>	<b>Std.Err.</b>	<b>b</b>	<b>Std.Err.</b>	<b>t(9)</b>	<b>p-value</b>
Intercept			3.29500	3.67061	0.897560	0.392797
XFC_trr	0.513454	0.110014	3.94500	0.84532	4.667150	0.001173
CANcover	0.647857	0.110014	-11.62900	1.97467	-5.888840	0.000232



Figure 1. Male representatives of *Hyalella* morphotypes encountered at Parque Estadual da Serra do Mar - Núcleo Santa virgínia. Legends: A) Morphotype 1; B) Morphotype 2.

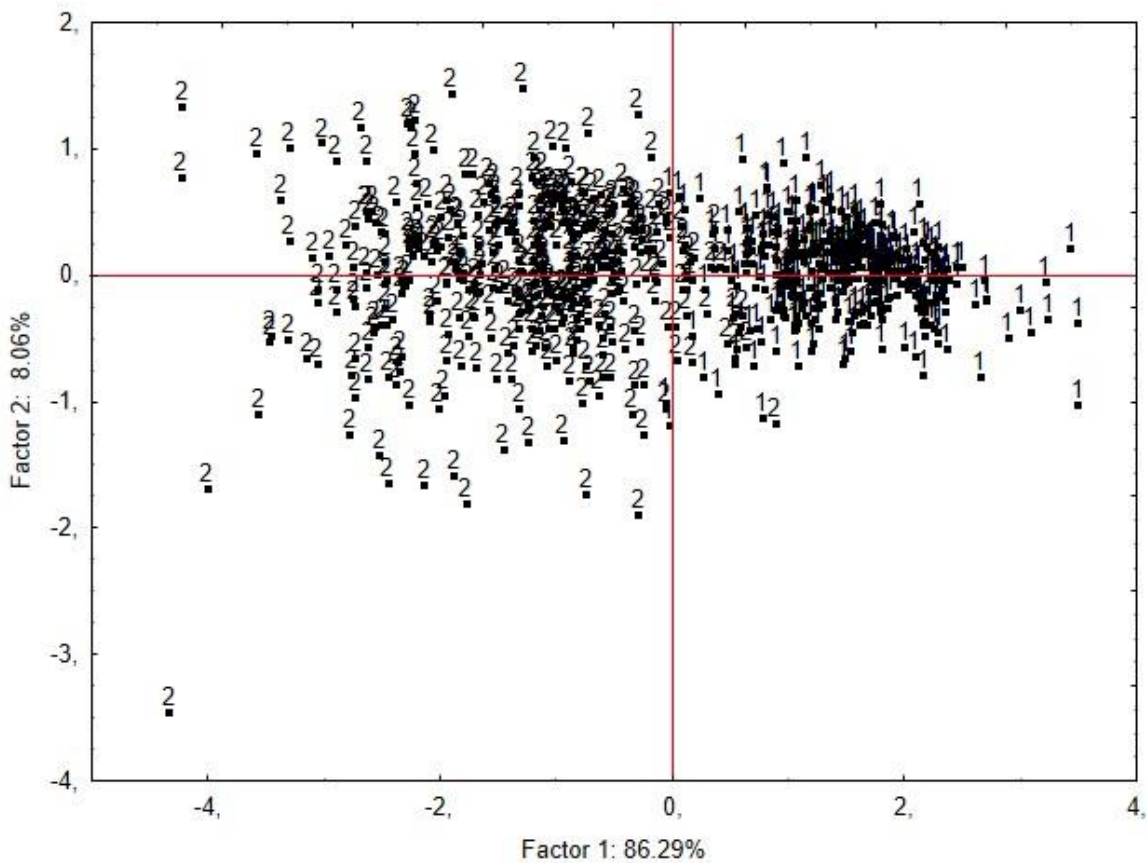
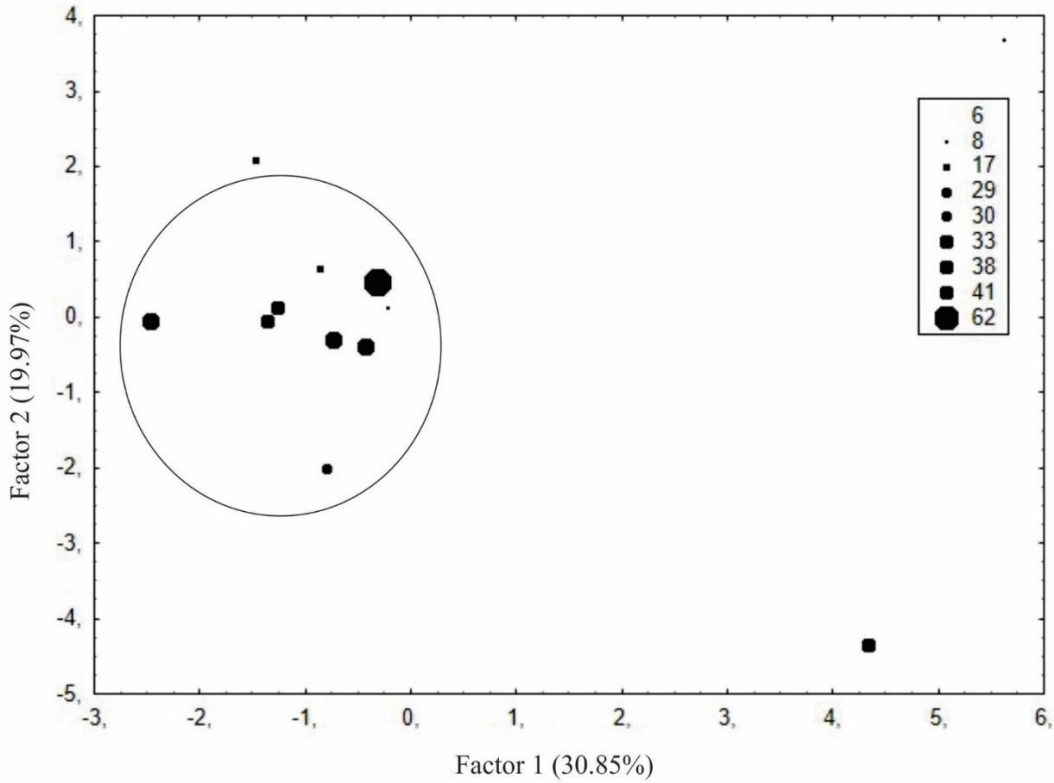
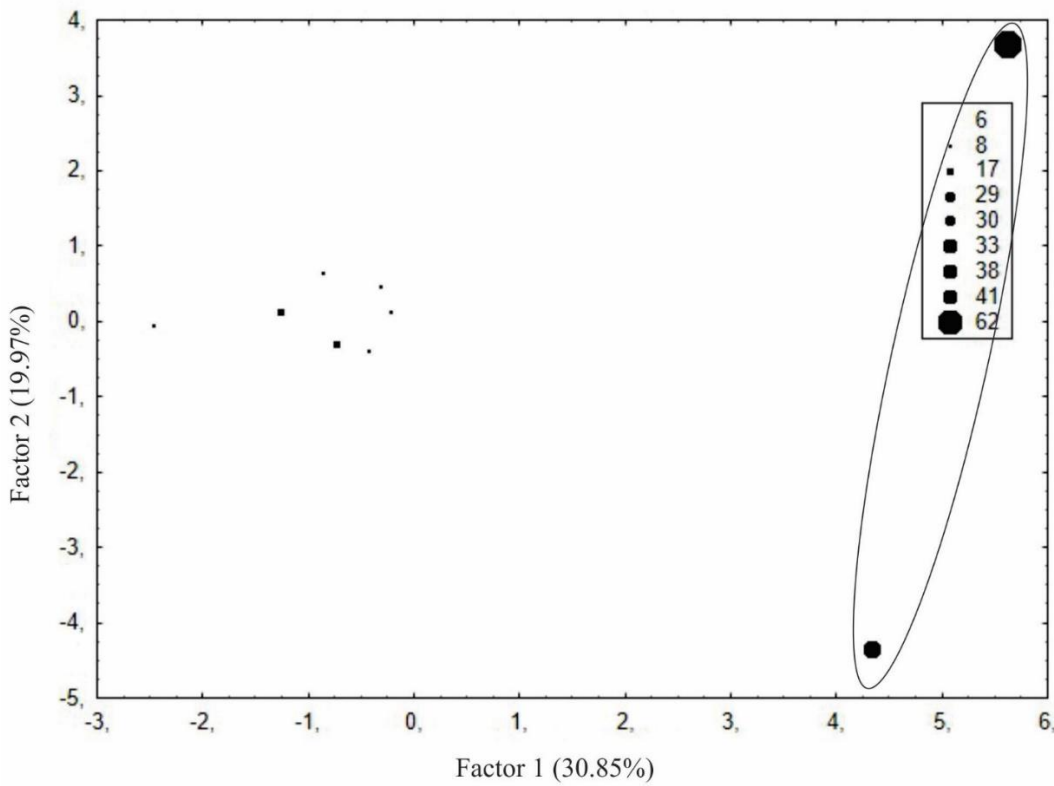


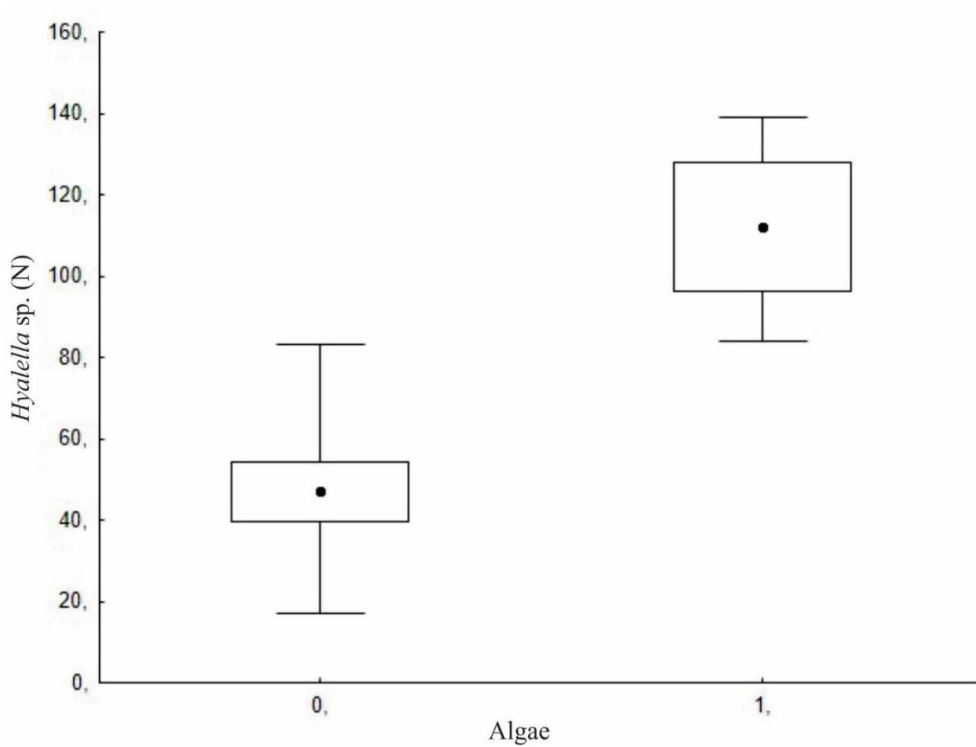
Figure 2. Principal Component Analysis plot for *Hyalella* morphotypes 1 and 2, based on morphological attributes. See table 3 for factors x variables correlations.



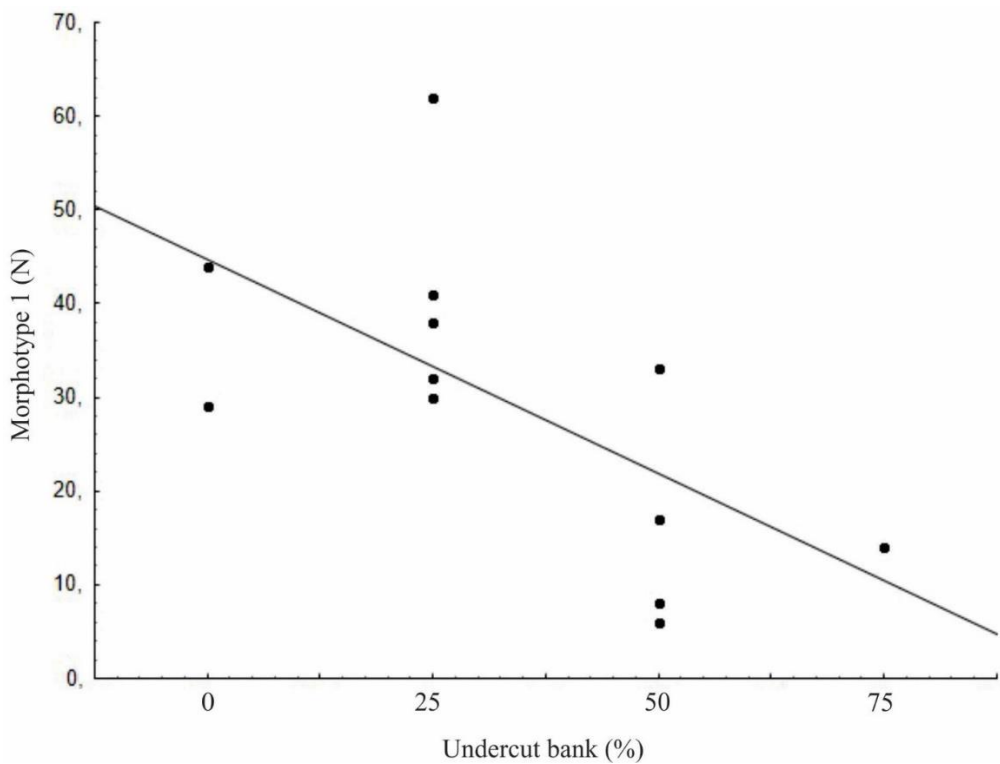
**Figure 3. Principal Component Analysis plot for sampled transects physical-habitat components. Bubble size is related to the abundance of *Hyalella* sp. morphotype 1. See table 5 for factors x variables correlations.**



**Figure 4. Principal Component Analysis plot for sampled transects physical-habitat components. Bubble size is related to the abundance of *Hyalella* sp. morphotype 2. See table 5 for factors x variables correlations.**



**Figure 5.** *Hyalella* sp. (morphotypes 1 and 2 combined) abundance related to algae presence. Barrs represent standard error.



**Figure 6.** Scatterplot of undercut bank (%) against *Hyalella* sp. morphotype 1 abundance (males, females and juveniles combined).

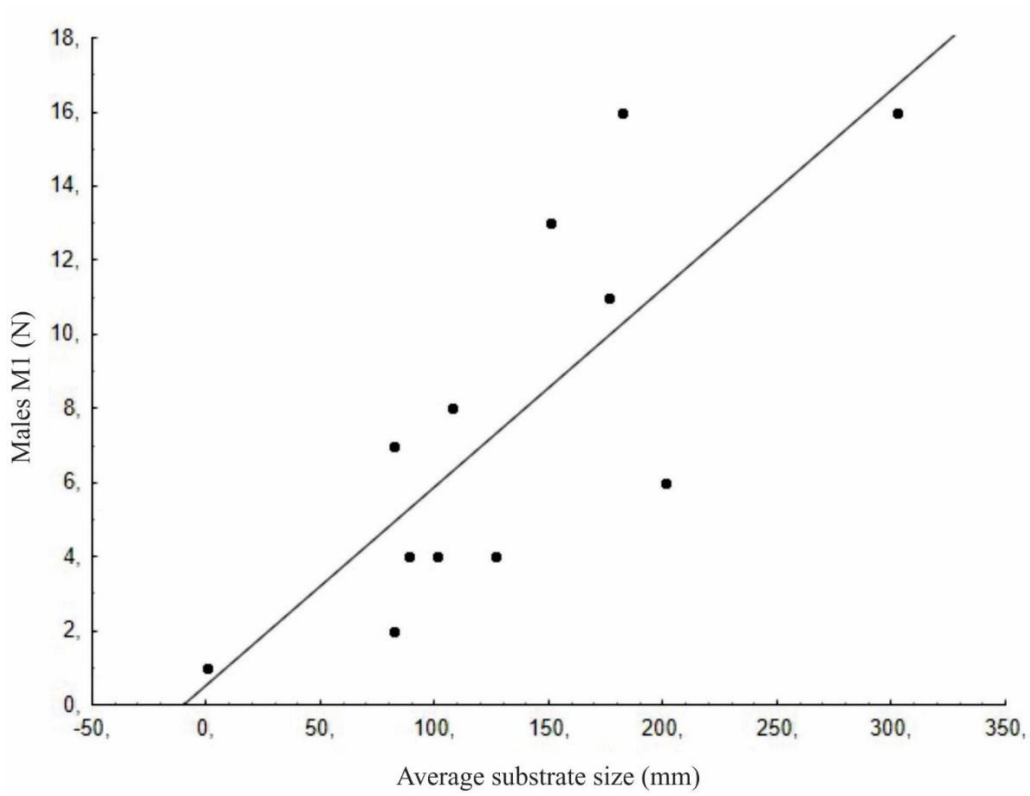


Figure 7. Scatterplot of substrate immersion against *Hyalella* sp. morphotype 1 male abundance.

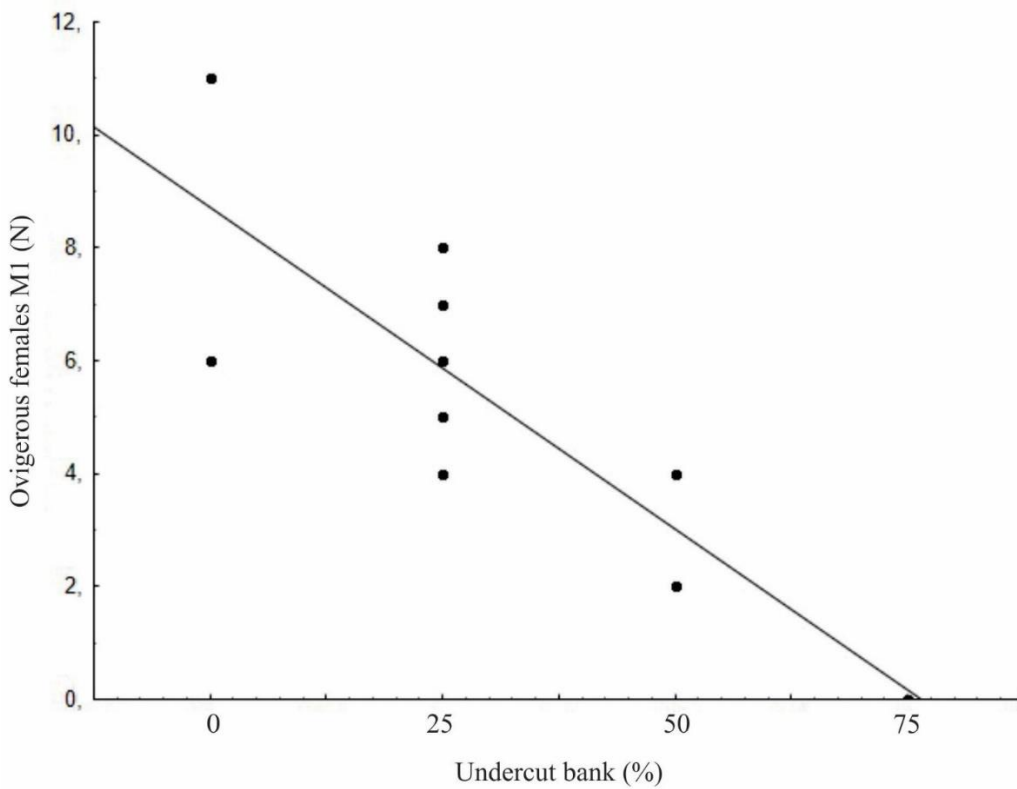


Figure 8. Scatterplot of undercut bank (%) against *Hyalella* sp. morphotype 1 ovigerous female abundance.

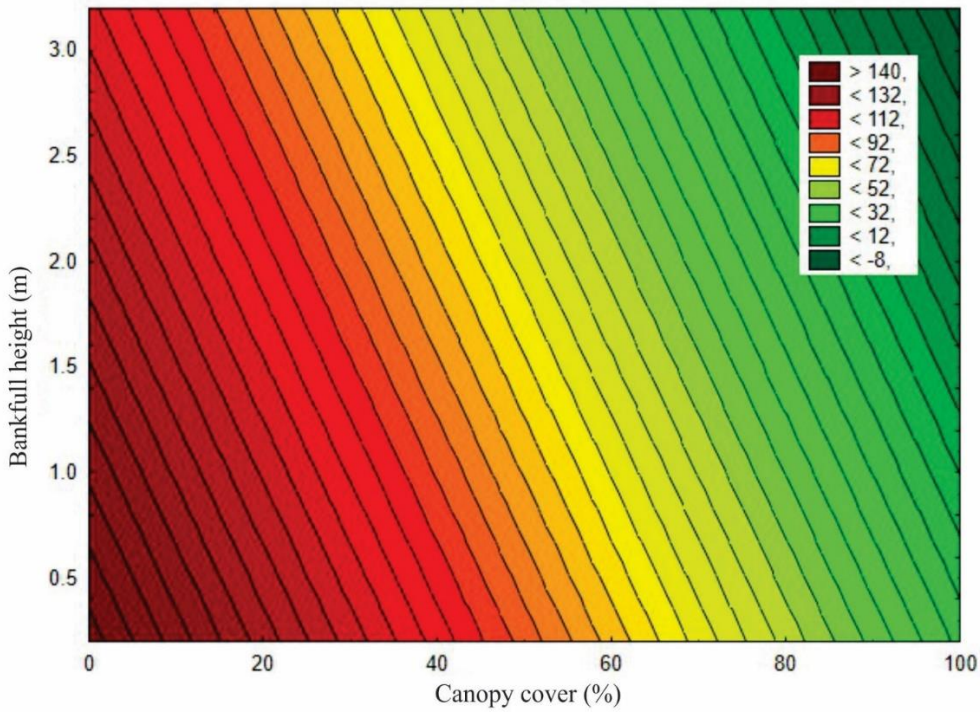


Figure 9. 2D contour plot of *Hyalella* sp. morphotype 2 abundance (males, females and juveniles combined) dependence on the interaction bankfull height and canopy coverage.

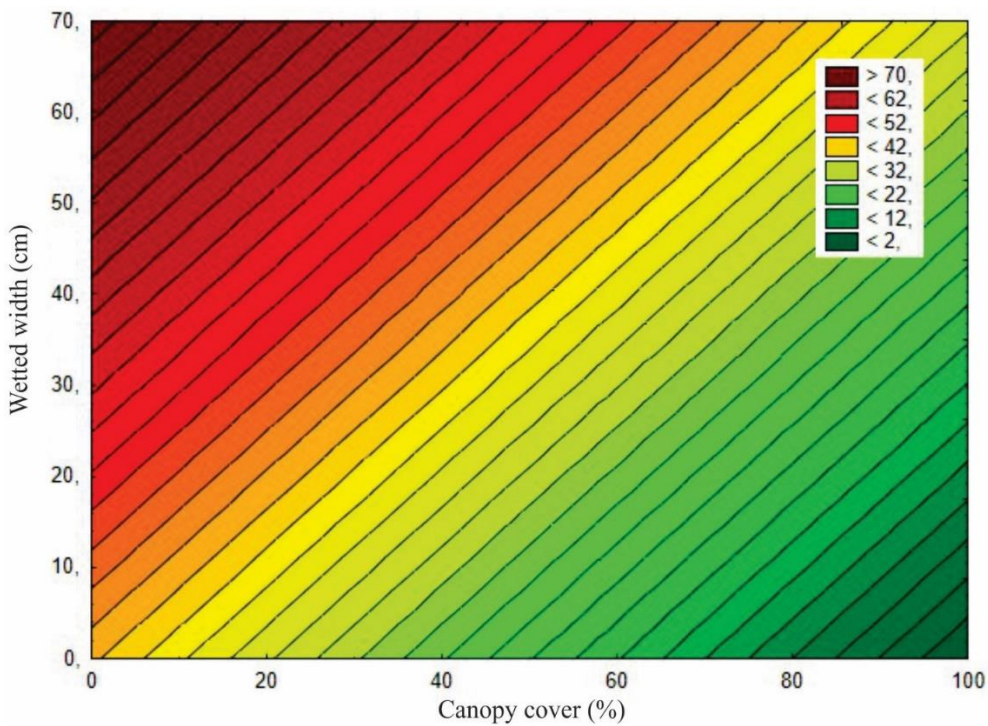


Figure 10. 2D contour plot of *Hyalella* sp. morphotype 2 male abundance dependence on the interaction wetted width and canopy coverage.

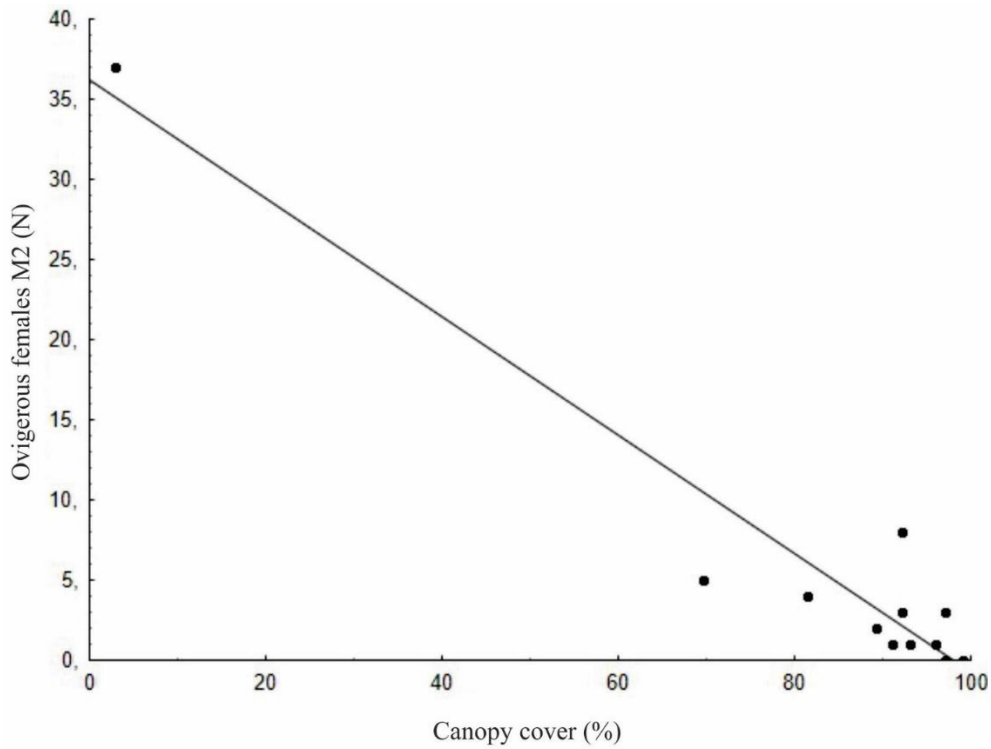


Figure 11. Scatterplot of canopy cover (%) against *Hyalella* sp. morphotype 2 ovigerous female abundance.

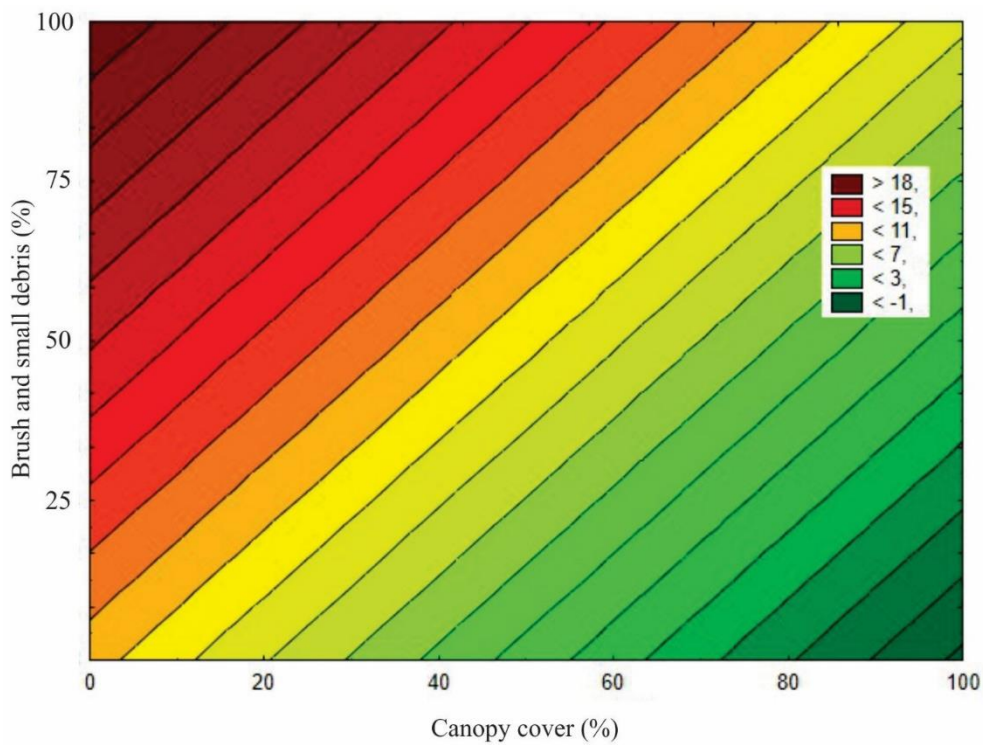
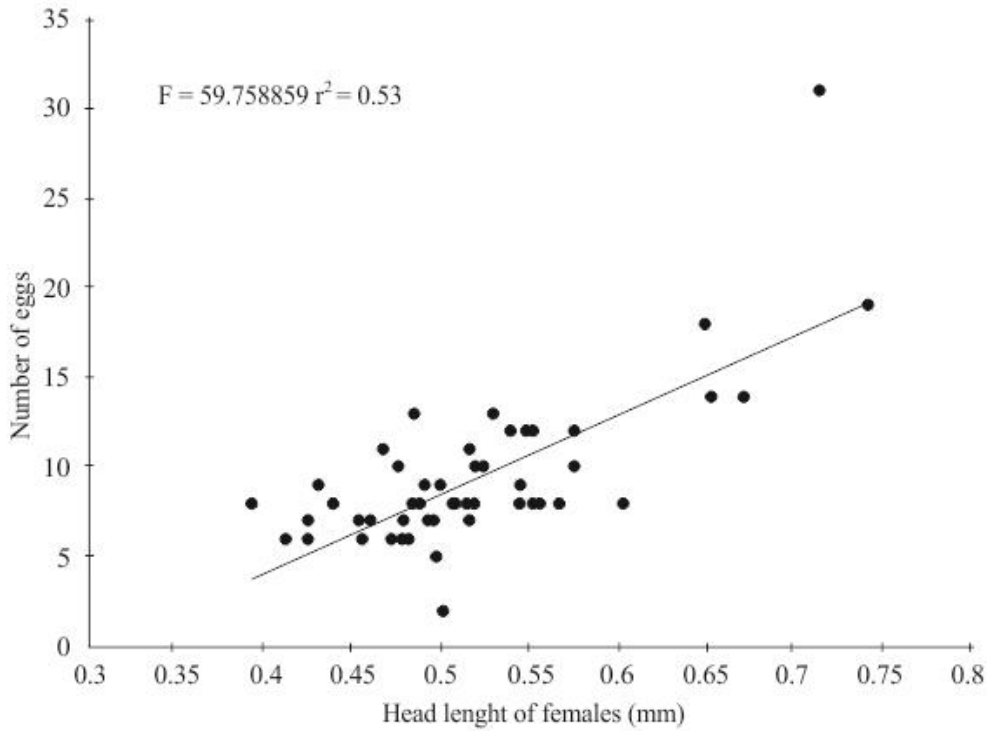
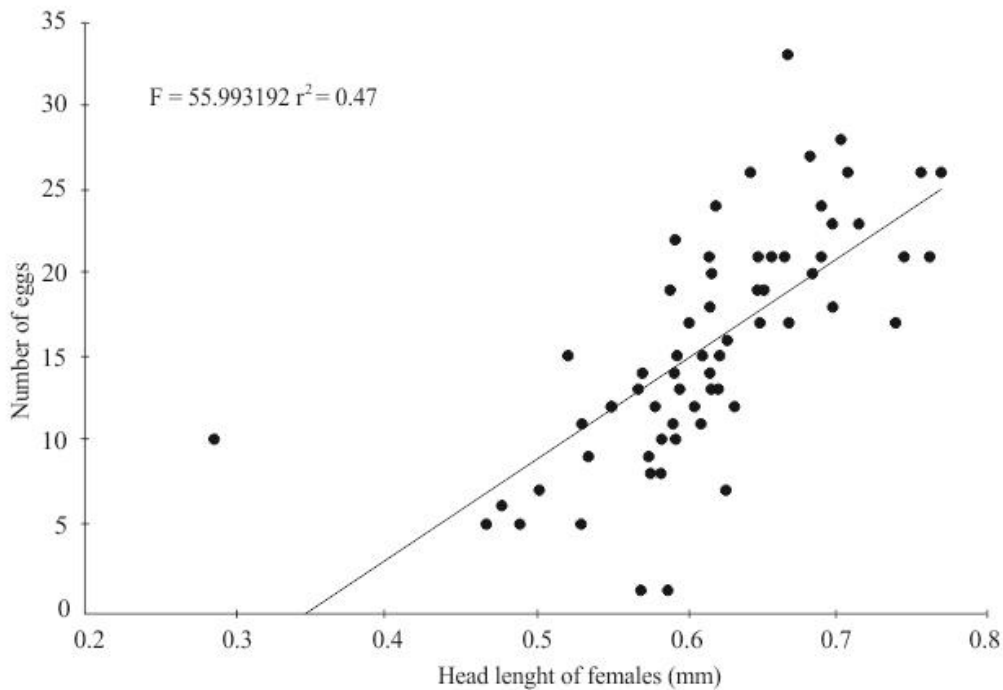


Figure 12. 2D contour plot of *Hyalella* sp. morphotype 2 juveniles abundance dependence on the interaction brush/small wood debris and canopy coverage.



**Figure 13.** Regression analysis between head length and number of eggs in the marsupium of ovigerous females *Hyalella* morphotype 1 from Parque Estadual da Serra do Mar - Núcleo Santa Virgínia.



**Figure 14.** Regression analysis between head length and number of eggs in the marsupium of ovigerous females *Hyalella* morphotype 2 from Parque Estadual da Serra do Mar - Núcleo Santa Virgínia.