Reproductive Variability in Hippolytid Shrimp Shape Morphotypes

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Shape morph-specific studies in hippolytid shrimps revealed significant results on their ecomorphology and evolutionary adaptations. Among the species of the genus Hippolyte, only one exhibits an unusual, sharp rostral dimorphism and has been used as an animal model for the investigation of mechanisms of the morph-specific adaptation: the intertidal Hippolyte sapphica. The species is endemic of the Central/Eastern Mediterranean basin and exhibits morph-A with a long dentate rostrum and morph-B with a short, juvenile-like one. The two morphotypes were recently confirmed to be conspecific, while offspring and morphological studies showed significant microevolutionary adaptations, which balance the disadvantage of the “rostral loss.” The present study aims to investigate the effect of such phenotypic variation on the reproductive traits of the species. We collected ovigerous females of H. sapphica in mixed (morph-A and morph-B) and unmixed populations (morph-A) along the species geographical range. We measured seven morphometric and maternal investment traits: carapace length, fecundity, embryo volume, egg density, female dry weight, brood dry weight, and reproductive output. Our results showed that ovigerous females were bigger in morph-A than in morph-B, whereas fecundity did not show any significant differences between the two morphotypes. High egg volume might be attributed to the latitudinal differences of our sampling sites compared to congenerics. Interestingly, the reproductive output was found to be bigger in morph-A specimens, suggesting that the maternal energy investment is selectively determined from the rostral presence/absence and the morphotype’s higher viability in the species populations.

1. Introduction

Marine decapods possess distinct reproductive strategies [1–4] that are mostly adapted to the prevailing conditions of their respective habitat [5–7]. The existence of trait plasticity related to environmental variations has been previously examined in species with wide geographical distribution [8–10]. However, little is known about the reproductive traits of small endemic caridean shrimps with restricted geographical range. Moreover, the reproductive features and output of decapods have a direct effect on the population dynamics in marine benthic assemblages they relate [11]. The size and quality of a clutch are highly associated with larval quality and survival; therefore, maternal investment could subsequently shape life-history characteristics of each species. Additionally, the specialization of reproductive strategies and the morphological polymorphisms of decapods allow the exploitation of optimum energy and food resources to the different taxa morphotypes [12, 13].

The genus Hippolyte is very diverse and consists of several small size marine shrimps, many of which are highly polymorphic [14]. Among the 37 species of the genus [14–19], four species demonstrate high morph and color variability, such as H. leptocerus [20], H. obliquimanus [9, 13], H. sapphica [20, 21], and H. varians [22, 23]. More specifically, H. obliquimanus contains an interesting intraspecific mosaic of
diversity, with color-specific types of individuals, which facilitate dietary needs into the different niches, and affect the species’ population dynamics [13, 24], while *H. sapphica* demonstrates morph-specific types of individuals, which have adaptive implications on the species morphology, population structure, and sex ratios [25, 26]. Moreover, representatives of the genus *Hippolyte* possesses an extremely intricate reproduction system, which exhibits distinct sexual mechanisms (e.g., hermaphrodite or gonochoristic) with variable traits among populations of different environmental conditions and geographical distributions [27–32]. Recently, Levy et al. [33] studied the mechanisms controlling the sex differentiation in animal model protandric shrimps, such as *H. inermis* and *Pandalus platyceros*, by means of transcriptomes, revealing the sex reversion processes via gene expression. A precise characterization and description of the genus’ sexual system is restricted to eight taxa ([17, 34, 35]; and references herein, [25, 36]), while information on the reproductive traits and strategies is limited [9, 27, 28, 37, 38].

*Hippolyte sapphica* is a dimorphic hippolytid of the sublittoral zone, inhabiting shallow, very sheltered lagoons and bays [20, 39, 40]. It prefers rich marine vegetation of *Zostera marina* and *Cystoseira* spp. [20, 21, 40] and is very abundant in *Cymodocea nodosa* meadows [41]. The species is endemic to the Central and Eastern Mediterranean Basins and has been recorded in the Northern Adriatic Sea ([42]; as *H. gracilis* [15]), the Ionian Sea [21, 25, 26, 40], the Aegean Sea ([43]: as *H. longirostris*; [44]: as *H. longirostris*; [15, 39–41]), and the Black Sea ([45]: as *H. prideauxiana*, 1967: as *H. inermis*). Moreover, two distinct morphotypes without intermediate forms are present along the species’ geographical distribution area: morph-A with a well-developed and dentate rostrum and morph-B with a juvenile-like, short rostrum, often referred to as “rostral loss” [20, 25, 39]. Morph-B has only been detected in the Amvrakikos Gulf (Greece) and the Venice Lagoon (Italy), whereas its sympatric morph-A has a wider distribution in Ionian, Aegean and Black Seas [25]. Previous studies suggested that the “rostral loss” could provoke positive and negative adaptive effects on the species population structure, sex ratios, and morphometry [25, 26]. More specifically, Liasko et al. [25] confirmed the parsimonious hypothesis that the “rostral loss” may be attributed to a single pair of alleles, with a complete dominance of allele b expressed in morph-B. Moreover, lab-reared and wild specimens showed a simultaneous appearance of sex appendages in both morphotypes; however, they were consistently developed later in wild female than in male specimens [25]. Additionally, morph-B reduces the viability and probability of egg-bearing among large females and exhibits higher propensity to become males [25]. The already studied sex ratios in both morphotypes support a gonochoristic status of *H. sapphica* ([25, 46]; under publication data on population dynamics of the two morphotypes). A comparatively morphometric study between the two morphotypes confirmed the hypothesis that morph-B females develop some compensatory morphological traits, substituting for the “rostral loss” [26]. This intraspecific dimorphic system affects mainly female morph-B individuals, by exhibiting a delayed development of non-ovigerous females and the enlargement of the body somites, the scaphocerite, and the telson. These ecomorphological adaptations could improve the hydrodynamic stability of the shrimp and compensate for the microevolutionary disadvantage of the “rostral loss.”

Here, we investigate the reproductive biology of the endemic *H. sapphica* across its restricted distributional range. We aim to examine the different patterns of maternity investment in species’ morphotypes and detect possible effects of “rostral loss” in fecundity, egg metrics, and reproductive output. We tested the hypothesis that morphological changes in morph-B ovigerous females, such as changes in abdominal segments related to the size of the brood chamber, may lead to functional adaptations in reproduction. Finally, we discuss our results in the light of published work on the maternal investment of the genus and highlight the variations of reproductive effort among hippolytid shrimps.

## 2. Materials and Methods

### 2.1. Sampling Sites and Sample Collection.

Ovigerous females were collected from four different sites, in the Ionian and the Aegean Greek coast, representing the two main zoogeographical regions of the species geographical distribution (Figure 1). Sampling was conducted during the summer months of 2011 and 2013 for the Ionian Sea site (St.1: Amvrakikos Gulf; mixed population) and during spring, summer, and autumn in 2017 and 2018 for the Aegean Sea sites (St.2: Vrasidas Bay, St.3: Fanari, and St.4: Lesvos Island; unmixed populations). The time difference between sampling periods is attributed to the lack of morph-B specimens in St.1 during 2017–2018, and older samples were used. The Amvrakikos gulf is a shallow and semi-enclosed embayment with limited water renewal from the Ionian Sea and coastal areas that are characterized by soft sediments and meadows of *Cymodocea nodosa*. The sampling stations of Vrasida, Fanari, and the island of Lesvos are located in coastal areas of the North Aegean Sea, with shallow muddy waters dominated by *Cymodocea nodosa* meadows. Agassiz trawl nets were used for most of the sample collection, while in St.1 (Amvrakikos Bay), all individuals were collected by means of a hand net (frame: 30 × 35 cm, mesh size: 2 mm), due to the special morphology of the artificial channel of the sampling site. The net was cast several times from the bottom to the surface of the channel along the aquatic vegetation. Samples were immediately preserved in ethanol (80%).

### 2.2. Laboratory Assessment.

Individuals were separated by morphotype through stereoscopic inspection of the rostra. Seven female morphometric and maternal investment traits were measured: carapace length (CL, mm), fecundity (F, number of eggs), embryo volume (EV, mm³), egg density (ED, g mm⁻³), female dry weight (FDW, g), brood dry weight (BDW, g), and reproductive output (RO). A precision scale (METTLER AJ150) was used for the weight measurements (ovigerous females and eggs), precise to the nearest 0.0001 g. Pictures of the carapace and egg mass of each individual were taken under a calibrated stereoscope (Nikon SMZ1500), equipped with a calibrated ocular...
micrometer, and measurements were taken on Image Pro software v.4.5.0. Carapace length (CL) was measured from the posterior margin of the ocular orbit to the posterior margin of the carapace [9]. The egg masses were isolated from the abdomen of the females, and their development was classified in three different stages: Stage I (uniform yolk), Stage II (visible eye pigment), and Stage III (visible eyes and abdomen) [47]. Additionally, the total number of eggs was counted to calculate fecundity. The smallest (a1) and the largest (a2) axes of each egg were measured and used to calculate the EV according to the formula $EV = (\pi \times (a1)^2 \times a2)/6$ [48]. Females and their egg masses were dried in an incubator (WTC binder) at 60°C for 24 hours and weighed (i) combined to acquire the FDW and (ii) separately to acquire the BDW. Only females with eggs in Stage I were used to calculate the RO by means of the formula $RO = BDW/FDW$, because egg loses are high in Stages II and III and RO calculations are biased [49]. ED was calculated by dividing BDW with EV. Voucher specimens were deposited and stored at the zoological collections of the Fisheries Research Institute, Nea Peramos, Greece.

2.3. Statistical Analyses. The hypotheses that morphological and reproductive traits were similar between morphotypes as well as sampling stations were tested using Permutational Multivariate Analysis of Variance (PERMANOVA). PERMANOVA was preferred to simple MANOVA because the data did not achieve multivariate normality (multivariate Shapiro Wilk normality test) that was tested using the R.test() function from the “mvnTest” package [50]. Additionally, the homogeneity of dispersions was tested using the betadisper() function in the “vegan” package [51]. The reproductive output was excluded from this analysis since RO data was not consistent and the metric created an unbalanced design that could not be analyzed. PERMANOVA was run with the Adonis() function in the “vegan” package with 999 permutations on the Bray-Curtis distance matrix, using either the Station as a factor with five levels or the Morphotype with two levels. Each question was addressed in separate analysis, since morph-B was only present in the Ionian Sea. Post hoc pairwise comparisons were executed using the pairwise.adonis() function in the “pairwiseAdonis” package [52]. The extent to which CL affects fecundity, egg volume and BDW was tested using linear regressions among all studied stations using the ggs Latino() function in the “ggpubr” package [53]. All statistical analyses were conducted in the R 3.5.1 (R Core Team, 2020; https://cran.r-project.org/), and plots were created using the package “ggplot2” [54]. All mean values in the text are followed by the standard error.

3. Results

In total, 392 individuals of H. sapphica ovigerous females were measured and analyzed. All the measured variables showed non-normal distribution. PERMANOVA showed statistically significant differences between H. sapphica morphotypes in St.1 ($F_{1,86} = 6.814, R^2 = 0.036, p = 0.008$); therefore, the two subgroups (morph-A and morph-B) were considered and treated separately in further statistical analyses. All stations showed statistically significant differences for the measured values ($F_{4,391} = 136.27, R^2 = 0.585, p < 0.001$). Post hoc analyses showed significant differences among the sampling stations with one exception between
St.2 (Vrasidas) and St.3 (Fanari). Carapace lengths ranged from 2.24 mm to 4.67 mm, while the mean CL size was 3.2 ± 0.5 mm. The bigger ovigerous females were recorded in St.2 (mean CL: 3.71 ± 0.5 mm) and the smaller in St.1 (mean CL: 2.79 ± 0.3 mm). In the mixed population (St.1), CL was generally bigger in morph-B than in morph-A. Additionally, size frequency distributions showed higher abundances of large females in the Aegean Sea (St.2, St.3, and St.4) with distributions peaks between 3.00 and 4.00 mm of CL, whereas smaller sizes were detected in the Ionian station (St.1, between 2.00 and 3.00 mm) (Figure 2).

3.1. Fecundity. Absolute fecundity ranged from 6 to 145 eggs (Table 1). Interestingly, all developmental stages showed similar trends of egg numbers among sampling sites, with the Ionian site (St.1) having the lowest and Fanari (St.3) the highest production (Table 1). Moreover, fecundity was significantly different between stations (\(F_{(3,382)} = 204.329, p < 0.0001\)) when controlled for CL, and it had a significant positive correlation to the covariate CL (\(F_{(1,382)} = 128.263, p < 0.0001\))(Figure 3). Vrasidas station (St.2) had the highest intercept (intercept = 43.244, \(p < 0.0001\)), and the lowest slope among stations (slope = 5.768, \(p < 0.0001\)). Fecundity did not show any significant differences between morph-A and morph-B (Figure 3).

3.2. Egg Metrics. Mean egg volume was 0.05 ± 0.020 mm\(^3\), with the lowest value in St.3 (0.038 ± 0.016 mm\(^3\)) and the highest in St.4 (0.063 ± 0.024 mm\(^3\)) (Table 1). Embryo volume increased with embryo stage. Moreover, embryo volume was not significantly different between morphotypes; it exhibited significant differences among stations (\(F_{(4,382)} = 21.937, p < 0.0001\)) when controlled for CL (Figure 4), and it had a significant positive correlation to the covariate CL (\(F_{(1,382)} = 35.38, p < 0.0001\)). Differences were mainly attributed to Vrasidas (St.2) that had a significantly lower intercept (intercept = 0.0879, \(p < 0.0001\)) and the highest slope (slope = 0.0365) of all stations (Figure 2). The mean egg density was 0.041 ± 0.066 g mm\(^{-3}\) and decreased with developmental stage (Table 1).

3.3. Reproductive Output and Brood Dry Weight. The mean value of the RO was 0.1796 (±0.066). For the mixed population, morph-A showed the higher RO (0.1898 ± 0.081) in comparison to morph-B (0.1537 ± 0.066). For the unmixed populations the higher mean value of RO has been recorded in St.2 (RO: 0.21 ± 0.06) and the lower in St.3 (RO: 0.1542
BDW was different among stations (intercept, \( F_{(4,382)} = 155.06, \ p < 0.0001 \)), while samples with different CL had different BDW (slope, \( F_{(4,382)} = 2.9932, \ p = 0.02 \)).

### 4. Discussion

The comparison of the reproductive traits of the *H. sapphica* mixed (morph-A and morph-B) and unmixed (morph-A) populations along the species distributional range showed differences in the fecundity investment and in the growth rate of ovigerous females. Ovigerous female body size was bigger in morph-A in the unmixed populations (Aegean sites: St.2, St.3, and St.4) than in the mixed population (Ionian site: St.1). According to Liasko et al. [25], morph-A becomes abundant with an increase in CL and the percentage of large ovigerous specimens is higher in this morphotype. Although morph-B exhibited larger ovigerous females than morph-A in the mixed population (St.1), the size frequency distributions revealed higher abundances in bigger size classes for morph-A in the unmixed population and higher abundances in smaller size classes for morph-A and morph-B in the mixed populations (Figure 2). Our results corroborate that morph-A appears to have higher viability in large ovigerous females [25]. Moreover, larger ovigerous females were found in Vrasidas (St.2, unmixed populations). This station is the only site with the appropriate ecological requirements for *H. sapphica* populations, as it...

### Table 1: Mean values of fecundity, embryo volume and egg density of *Hippolyte sapphica* ovigerous females at four sampling stations. NI: number of individuals; Min: minimum; Max: maximum; SD: standard deviation.

<table>
<thead>
<tr>
<th>Stages</th>
<th>NI</th>
<th>St.1 (Amvrakikos Gulf) Morph-A</th>
<th>St.1 (Amvrakikos Gulf) Morph-B</th>
<th>St.2 (Vrasidas)</th>
<th>St.3 (Fanari)</th>
<th>St.4 (Lesvos Isl)</th>
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<tr>
<td></td>
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<td>Fecundity Mean (±SD) Min Max</td>
<td>Fecundity Mean (±SD) Min Max</td>
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<td>Fecundity Mean (±SD) Min Max</td>
</tr>
<tr>
<td>I</td>
<td>80</td>
<td>18 ± 10 6 65</td>
<td>23 ± 17 7 91</td>
<td>65 ± 21 13 107</td>
<td>72 ± 27 32 127</td>
<td>38 ± 13 21 111</td>
</tr>
<tr>
<td>II</td>
<td>24</td>
<td>15 ± 8 6 38</td>
<td>27 ± 15 10 55</td>
<td>68 ± 13 45 80</td>
<td>84 ± 29 45 145</td>
<td>43 ± 12 21 68</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>16 ± 11 8 32</td>
<td>34 ± 18 11 51</td>
<td>58 ± 13 45 80</td>
<td>59 ± 27 21 99</td>
<td>43 ± 12 21 68</td>
</tr>
</tbody>
</table>

The mean BDW was 0.0013 (±0.001) g. Stages I and III exhibited the same BDW value (0.0013 ± 0.001 g), whereas Stage II was slightly lower (0.0011 ± 0.001 g). BDW was significantly different between stations when controlled for CL, as it had a significant positive correlation to the covariate CL (\( F_{(1,382)} = 126.77, \ p < 0.0001 \); Figure 5).
is a sheltered and shallow bay with well-established *Cymodocea nodosa* meadows [20, 41]. Therefore, the larger size of individuals could be attributed to the food adequacy and to the lack of exposure due to the extended and permanent seagrasses’ presence usually found in the Aegean sites. Conversely, the site in Amvrakikos Gulf (St. 1, canal connected to the gulf) is characterized by poor aquatic vegetation and the absence of seagrass meadows.
Absolute fecundity ranged from 6 to 145 eggs in *H. sapphica*, which is placed within the typical range of the genus ([9, 55] and references herein; [37]). Lower egg production has been reported for *H. nicholsoni* [56] with 25 eggs per ovigerous female, while higher egg production (64-187 eggs/ovigerous female) has been reported for *H. obliquinaurus* [9, 57]. *Hippolyte zostericola* demonstrated similar egg production with *H. sapphica* (104-147 eggs/ovigerous female) [56, 58] at similar geographical latitudes. Fecundity did not show any significant differences between morph-A and morph-B (Table 1), suggesting that the dimorphic system of the rostral presence/absence did not affect egg production. Among morph-A populations, higher mean fecundities were recorded in St.2 and St.3 (Table 1), which could be attributed to the favorable environmental conditions (shallow, shelter, and seagrasses’ habitats) [41, 59, 60], and the presence of larger female. Egg staging showed similar trends in embryo number between morphotypes and among stations, indicating that the egg loss is low and similar between morphotypes and sites.

Mean egg volume is higher in *H. sapphica* compared to congeneric species [9, 55–58]. Egg volume is an intraspecific biometric marker with high variability and is usually correlated to the ovigerous female size, the duration of embryonic development, larvae size, etc. Decapod fecundity and egg volume are highly correlated to geographical latitude and temperature, with high egg number and small egg volumes being reported in lower latitudes and higher temperatures [9, 61]. Hippolytid tropical species show lower egg and embryo volumes [55], whereas there is a lack of information for species reported in temperate regions. Moreover, hippolytid interpopulation comparisons confirmed that higher mean fecundities have been recorded in lower latitudes [9]. The high egg volume observed in *H. sapphica* could be attributed to (a) the higher latitudes of the sapling sites, which ranged from 39°12’52.24″N (St.4) to 40°57’50.92″N (St.3), and (b) the lower mean surface water temperatures, which varied from 18.43°C to 25.4°C (St.1: 25.4°C, St.2: 19.55°C, St.3: 18.43°C, and St.4: 20.03°C). Egg volume did not show any significant differences between the two morphotypes, while egg density was significantly lower in morph-B, due to its negative correlation with the brood dry weight and the female size in morph-B (Table 1, Figure 5). No differences were detected in the duration of

Figure 5: Linear regression between carapace length and brood dry weight among study sites and morphotypes for *Hippolyte sapphica* ovigerous females.
embryonic development or in the hatched larvae size between morphotypes [21, 25], justifying the observed similarities in measured egg volume.

The reproductive output for *H. sapphica* was high (0.1796 ± 0.066) and similar to *H. obliquimanus* (RO: 0.13-0.17; [9]). Between the two morphotypes, morph-B invested lower energy in embryo production (RO: 0.1537 ± 0.066) than morph-A. Liasko et al. [25] showed that this intra-specific rostral dimorphic system offers viable advantages in morph-A which are connected to the life strategy of the species. Morph-A specimens demonstrated high propensity to become females (A/B in females ratio: 1: 0.63), while morph-B specimens to become males (A/B in males ratio: 0.49: 1) [25]. The current comparison of the reproductive metrics of mixed and unmixed *H. sapphica* populations revealed that the mean fecundity does not demonstrate differences between morphotypes. However, the reproductive output suggested that the energetic investment is oriented towards female morph-A individuals (higher mean values of RO for morph-A (mixed population): 0.1898 ± 0.081, and (unmixed population): 0.2097 ± 0.06). Thus, the RO does not serve as a direct reproductive index connected to fecundity, but rather as an indirect reproductive index of body robustness and good condition of the morph-A sub-population. Similarly, *H. obliquimanus* color morphotypes were associated with robust morphology and high mobility in water media [12]. Therefore, shape and/or color morphotypes could induce morph-specific life-styles. Brood production is a relatively stable and fixed reproductive character typical for each species, which is not usually affected by microevolutionary processes. Despite similar values of fecundity between morphotypes and habitats, the sex ratios were defined by a female-biased system which explains the habitat use of the species and its ecological adaptations [12]. Moreover, morphometric analysis of both *H. sapphica* morphotypes showed that morph-A females develop more rapidly and they reach an earlier reproductive age than morph-B, a type which is male-oriented [26]. A detailed examination of the seasonal demography of the species’ mixed and unmixed populations will demonstrate specific energetic adaptations through the size frequency distributions, the median size of maturity, and the seasonal sex ratios.

Our study shows that the present intraspecific rostral dimorphic system regulates selectively the reproduction and the eggs’ metrics through unequal growth investment between the two morphotypes in the mixed and the unmixed populations of *H. sapphica*. This suggests a selection which acts on the sex ratios of the species’ morphs, producing a female-biased morph-A with high reproductive energy investment, and a male-biased morph-B. This strategy, in combination with the morphological adaptations of the morph-B individuals, could reflect differential life history adaptations of *H. sapphica* morphotypes to the “rostral loss” condition. The molecular mechanisms and pathways could provide important insights for understanding crustacean adaptation processes; however, they have not been investigated in *H. sapphica*. Our conclusions summarize how maternity investment, morphological traits, and growth parameters are inter-dependent when a main structure, as rostrum, is missing.

**Data Availability**

Data is available on request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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**References**


