# Are the shrimps Halocaridina rubra and H. palahemo simply different morphotypes

of the same species?

by

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## Abstract

The shrimp genus *Halocaridina* (family Atyidae) contains only two species. *Halocaridina rubra* is found throughout Hawaii and usually feeds benthically. *H. palahemo* has only been found in one pool and usually feeds pelagically. The two species are similar but have several different physical characteristics that may be related to their feeding modes. It has been suggested that they are the same species which develops different morphology under different feeding conditions. In this experiment, *H. rubra* was exposed to two different kinds of food sources (benthic and pelagic algae), and the physical changes of the carapace, rostrum, chelae, dactyl, setae and carpus of the first and second pereiopods were tracked. The hypothesis was that the shrimp which consistently feed on pelagic algae would develop characteristics similar to *H. palahemo* including: increased inflation of carapace, shortened rostrum, decreased inflation of the chelae and lengthening of the carpus, dactyl and setae of the first and second pereiopods.

Although variability was observed in these shrimp over the six months of this experiment, the variability was mostly not in the pattern that would be expected if the pelagic group was transitioning to the morphology seen in *H. palahemo*. This experiment did not either fully support or refute the hypothesis that *H. rubra* and *H. palahemo* are simply different morphotypes of the same species based on feeding mode. The experiment did, however, suggest modified conditions that will need to be implemented to further test the hypothesis.

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#### **INTRODUCTION:**

Most species have a distinct morphology. Particular morphologies are so characteristic of species, that morphology is one of the primary ways in which species are often distinguished. However, there are some instances in which some species can make substantial changes to their morphology. Cyclomorphosis, for example, is a phenotypic polymorphism in which animal shape may cycle into several different alternate forms due to seasonal or environmental factors. Phenotypic responses such as these have been observed in a surprising variety of creatures including barnacles (Lively, 1986), algae (Van Donk et al., 1999), amphibians (McCollum and Leimberger, 1997) and fish (Bronmark et al., 1999).

One of the best known examples of cyclomorphosis is the formation of helmets and spines in *Daphnia*, a small branchiopod crustacean common in freshwater habitats. The growth of a long, helmet-like projection on the top of the head and an elongation of the tail spine occur seasonally (Figure 1). These changes are thought to serve as a defense against predators, including fish and invertebrates (Laforsch and Tollrian, 2004). Many experiments have shown that these cyclomorphic changes become much more pronounced when predators are present (Laforsch and Tollrian, 2004). Multiple induction factors of the cyclomorphosis, such as turbulence or kairomones released by predators, often have synergistic effects and may stimulate a larger helmet or spine than a single induction factor does (Tollrian and Laforsch, 2006).



Figure 1: The helmeted (top) and non-helmeted (bottom) morphs of Daphnia covering the first eight instars. The helmeted morphology is induced by the presence of predators. HL = Helmet Length. BL = Body length. SL = Spine Length (Photo from Laforsch 2004)

The ability to change morphology in this way based on environmental cues may require a large genetic investment. Thirty-six percent of *Daphnia's* genes, for example, have no detectable homologs (Ebert, 2011). Ebert suggested that many of the species' unusual genes may contribute to their ability to react to environmental conditions by changing their morphology.

Studies of cyclomorphosis are generally conducted on an intergenerational time scale for *Daphnia*. These changes mostly likely occur between generations, which may indicate that a maternal factor is involved which can be passed on to offspring. However, if these changes take place in a single individual over the course of several molts the morphogenesis would likely indicate environmental induction of different gene expression instead. It has been suggested that these changes may be due to epigenetic regulation (Harris, et al, 2012).

While cyclomorphosis is well known in small, short-lived branchiopod crustaceans such as *Daphnia*, few environmentally-induced morphological changes have been reported in other groups of crustaceans such as crabs and shrimp. Atyidae is a family of small shrimp that usually live in freshwater caves, streams, or pools. The different species within the family typically live in different habitats and have one of two basic feeding methods: scraping or filtering (Felgenhauer and Abele, 1985). In the scraping feeding mode, which is the mode characteristic of most species in the family, the shrimp use enlarged chelae (claws) to scrape biofilms off solid surfaces. The shrimp then consume the algal cells scraped off the surface. Atyid shrimp species which use the filtering mode, on the other hand, typically have slender chelae with a fan of long setae attached. These shrimp use the setae to sweep through the water, either by holding their chelae up into the current if they live in a stream or by actively swimming if they live in still water. The pelagic algae they capture are consumed for food.

*Halocaridina* is a genus in the family Atyidae with very limited and unusual distribution (Figure 2). The genus only occurs in the Hawaiian island chain, and is found only in anchialine pools along the coast. Anchialine pools are inland bodies of water which are influenced by the nearby oceans via underground cracks or passages. The water in anchialine pools is brackish, showing both freshwater and marine influence. Further, the pools are affected by the tides in the nearby ocean, confirming the underground connection. Such pools are found in a variety of locations along the coast of the Hawaiian islands where porous basalt flows have encountered the ocean (Figure 2). Their morphology varies from shallow, open exposed pools to deeper pits and even to caves.

The genus *Halocaridina* has only two described species. *Halocaridina rubra* (Holthus, 1963) (Figure 3) is found throughout several islands of Hawaii (Craft et al., 2008) (Figure 2). This species usually feeds by scraping on the benthic algae or vegetation that fall into the pools. Some distinguishing physical characteristics of this species (Table 1) are: 1) the carapace is moderately inflated; 2) the rostrum reaches well beyond the eyes; 3) the





Figure 3: *Halocaridina rubra* on rocks which are covered in encrusting algae.

Table 1: Contrasting morphological characteristics of *H. rubra* and *H. palahemo* as listed in Kensley and Williams, 1986.

	<u>H. rubra</u>	<u>H. Palahemo</u>
Carapace:	Moderately inflated	Strongly inflated
Rostrum:	Reaching well beyond eye to antennular peduncle article 2	Just reaching to ends of eye
Chelae:	Strongly inflated	Barely inflated
Carpus of pereiopods 1 and 2:	Shorter or Subequal to Chelae	Equal to or longer than Chelae

chelae are strongly inflated; and 4) the carpus of both the first and second pereiopods is than or subequal in length to the chela of those pereiopods (Kensley and Williams, 1986).

*H. palahemo* (Kensley and Williams, 1986) has only been found in one cave (Figure 2). This species tends to swim more than *H. rubra*. Some of the distinguishing features of *H. palahemo* (Table 1) are: 1) the carapace is strongly inflated; 2) the rostrum reaches just to the end of the eyestalks; 3) the chelae are barely inflated; and 4) the carpus of the first and second pereiopods is equal to or longer than the chela (Kensley and Williams, 1986).

*H. rubra* and *H. palahemo* share a variety of features, but have several physical characteristics that are clearly different. Both species show variation in these characteristics which may be a result of different environmental conditions (Bailey-Brock and Brock, 1993). Some have suggested that when the shrimp feed on benthic algae they may have a morphology similar to that of *H. rubra*. However, while feeding on pelagic algae, the morphology may come to resemble that of *H. palahemo* (Richard Sternberg, personal communication). This suggests that *Halocaridina rubra* may be capable of adjusting its morphology due to environmental conditions in a manner similar to cyclomorphosis, and that *H. palahemo* may simply be a morph of *H. rubra*.

## Hypothesis:

In this experiment, *H. rubra* was exposed to two different kinds of food sources (benthic and pelagic algae). Physical changes to the carapace, rostrum, chelae, dactyl, setae and carpus of the first and second pereiopods were tracked and recorded. The hypothesis was that the shrimp which consistently feed on pelagic algae would develop characteristics similar to *H. palahemo* including: increased inflation of carapace, shortened rostrum, decreased inflation of the chelae and lengthening of the carpus, dactyl and setae of the first and second pereiopods (Table 2). The shrimp that were feeding on the benthic algae, on the other hand, would continue to show resemblance to *H. rubra* including: less inflation of carapace, longer rostrum, strong inflation of the chelae and a short carpus, dactyl and setae of the first and second pereiopods. These changes were expected to occur within the first individuals subjected to these conditions and to be more pronounced in the second and succeeding generations.

Table 2: Predictions of changes expected in the pelagic shrimp group.

Predictions based on previous papers:

- In the pelagic shrimp, the ratio of carapace length to height will become less (more inflated)
- In the pelagic shrimp, the length of rostrum/length of carapace will decrease
- In the pelagic shrimp, the rostrum will not project past eyestalks
- In the pelagic shrimp, the length/width of the palm of the first propodus will increase (become long and thin)
- In the pelagic shrimp, the length/width of the palm of the second propodus will increase (become long and thin)
- In the pelagic shrimp, the length of the carpus/ length of the palm of the first propodus will increase
- In the pelagic shrimp, the length of the carpus/ length of the palm of the second propodus will increase

Further predictions based on anecdotal data or logic:

- In the pelagic shrimp, the setal length/spread on the first propodus will increase
- In the pelagic shrimp, the setal length/spread on the second propodus will increase
- In the pelagic shrimp, the setal length/dactyl length on the first propodus will increase
- In the pelagic shrimp, the setal length/dactyl length on the second propodus will increase
- In the pelagic shrimp, the setal length/palm length on the first propodus will increase
- In the pelagic shrimp, the setal length/palm length on the second propodus will increase
- In the pelagic shrimp, the dactyl length will increase on the first propodus
- In the pelagic shrimp, the dactyl length will increase on the second propodus
- In the pelagic shrimp, the shrimp will swim more

#### **MATERIALS AND METHODS:**

## **Experimental Setup:**

Approximately 300 shrimp identified as *H. rubra* (which are called "opae-ula" in Hawaiian) were obtained via <u>http://www.hawaii-opaeula.com/</u>. They arrived in 14 ppt (parts per thousand) seawater and were allowed to warm up to 25°C before being added to the holding tank. They were held for 6 days in a five gallon (19 liter) aquarium at 25 ± 2°C, in brackish (16-17 ppt) seawater, made using Bio Sea salt<sup>®</sup>. A 12 hr/day:12 hr/night cycle was provided via a timed incandescent light. Filtration and aeration were provided by a sponge filter and air pump. The shrimp were fed algae pellets (Wardley Algae Disks<sup>®</sup>) once during the holding period, along with the encrusting algae on the rocks that they were shipped with.

Each of the two experimental tanks was a 51 by 36 by 31 cm (10 gallon) aquarium. Both tanks had been split down the middle with a 200 µm mesh, nylon screen siliconed in place with an acrylic border (Figure 4). A pump installed in the side which contained the adults (side G0, generation 0) moved water from the adult side of the screen to the opposite side. This water moved slowly back through the central screen and circulated throughout the tank. The pump intake was attached to a funnel which was covered with a 2 mm mesh screen at its large end (9 cm in diameter) to avoid disturbing the shrimp by suction. The purpose of the pump intake screen was not only to avoid disturbing the



The experimental tanks were 51 x 36 x 31 cm (10 gallon) aquaria. Each tank (BO) was restricted and split in two near the end. The pelagic tank had no rocks, plus the tubing was split farther water slowly moved back to side G0 through the central screen. To prevent adult shrimp from being sucked into central screen (CS). A pump (P) on side G0 sucked in water and pumped it through tubing (T) to side G1. The the pump, the pump intake was attached to a funnel (F) with the top covered by a coarser mesh (1.7 x 1.1 mm) intake screen (IS). The benthic tank also had algae-covered rocks (R) along the bottom plus the tubing outlet was divided down the middle into a G0 (generation 0) and G1 (generation 1+) section by a fine 200 µm mesh back and had a separate outlet (PO) directed toward the middle of each side of the tank. Figure 4: Experimental tank setup.

adults, but also to separate the adults (generation 0) from their offspring (generation 1+) by drawing the larvae through the intake screen, through the pump and depositing them on the opposite side of the tank. The fine mesh central screen would then prevent them from returning. By separating the generations, intergenerational changes would become clear.

The two experimental tanks each had a slight modification to this basic design to fit the purpose of the tank. Tank 1, the benthic tank, had the flow rate reduced via a reduction and a T-fitting after the pump on the side meant to catch generation 1 (side G1), so the outflow velocity and turbulence were reduced (Figure 4). The natural algal-covered rocks that the shrimp had been shipped with were placed on the bottom of the benthic tank for food and habitat. Shrimp in this tank were also initially fed algal pellets once or twice a week, with any remaining pellets removed before adding additional ones. Their main food, and their only food after the pellets were removed, was algae scraped from the rocks as is characteristic of *H. rubra*.

In tank 2, the pelagic tank, it was necessary to maintain water movement in order to keep the powdered algae food suspended. For this tank to achieve this objective, the exit from the pump was split into two streams, one for each side of the tank (G0 and G1). Each outflow stream directed a gentle but constant stream of water over the floor of the aquarium to provide the water movement necessary to keep the algae in suspension. To discourage the shrimp from spending time on the bottom of this tank, no rocks were placed in it and the bottom was bare glass. Powdered *Spirulina* algae, which are fine particles and remained mostly in suspension, were added to the pelagic tank 2-3 times a week. On the same schedule, any algae that had settled to the bottom of the tank were removed by suction, plus once a week the tank was scrubbed to remove any algal growth. After the fluorescent light was added (May 24, 2012), in addition to the incandescent light, the tank scrubbing became twice as frequent, as the light induced more algae growth. The goal for this tank was to provide a primarily pelagic food source and relatively inhospitable surfaces so the shrimp would be encouraged to spend as much time as possible swimming and to gather their food from the water column as is characteristic of *H. palahemo* rather than scraping it from the bottom. A partial (approximately 1/3) water exchange was conducted on each tank at one month intervals for the first two months before concluding that the partial water changes every week would be adequate for water exchange. At this time, a larger amount of water was removed from tank 1 to remove excrement.

At the beginning of the experiment (Month 0, February 1, 2012, Table 3), 145 shrimp with the representative size range between 1.9 and 2.74 mm present in the shipment were added to each tank. As the experiment progressed, it became evident that some adjustments were needed for the tank setup. First, some of the smallest shrimp fit though the 2 mm mesh of the pump intake funnels and were deposited on the G1 side of the tank. To remedy this, the pumps were shut down on the first day and a finer (1.7x1.1 mm) mesh was added to the pump intake funnels. Second, some of the shrimp in the benthic

Table 3:	Month of the	experiment and	corresponding	t date of the	measurements.

Month	Date
0	February 1, 2012
1	March 2, 2012
2	April 3, 2012
3	May 1, 2012
4	June 5, 2012
5	July 6, 2012
6	August 1, 2012

tank appeared to wait until the supplied algal pellets disintegrated, then consumed this softer material. To encourage benthic scraping, food pellets were no longer added after April 12 (between months 2-3). This allowed the shrimp to feed exclusively by scraping algae from the rocks they were shipped with. Lastly, as they grazed solely on the algae covered rocks, the alga population began to decrease on the rocks. Additional florescent light was provided in both tanks to compensate and encourage algal growth.

#### **Behavioral Measurements:**

The swimming activity of at least 10 shrimp from each experimental tank was monitored once a month for a half hour period to determine if the different environmental and feeding conditions in tanks 1 and 2 had any effect on behavior. For this experiment, the shrimp were transferred to a small aquarium (10x10x5 cm) without food. After a 10 minute acclimation period, a video was taken for 30 minutes while the investigator left the room. Studies have shown that the shrimp do not appear to have a diurnal cycle in an environment without predators (Carey et al., 2011). However, the time of day was standardized at 10 am to eliminate any concerns. At each minute mark, the number of shrimp swimming was recorded. These were converted to proportion, arcsine transformed, and compared among months and between treatments (benthic and pelagic) by ANOVA. After testing, these shrimp were returned to their respective aquaria or were used for the morphological measurements (see below).

#### **Morphological Measurements:**

Each month, 8-10 shrimp were removed from each experimental tank for measurement of changes in morphology. Before being measured, these shrimp were euthanized in 70% ethanol. Since the dimensions of the carapace, rostrum, chelae, and carpus of the first and second pereiopods are the main distinguishing characteristics listed for the two species (Table 1); these features were the main focus of this study. Several other measurements, such as the length and width of the setal bristles used for gathering food, based on general statements in the literature and on logical inferences about changes that could be expected with the different feeding styles were added. The height and length of the carapace were measured (Figure 5). The height was measured near the middle at the greatest height. The length was measured from just behind the eye straight back to the end of the carapace. The height/length ratio was used to express the degree of inflation of the carapace. The length of the rostrum was measured (Figure 5). The rostrum length was measured from the edge of the carapace next to the base of the eyestalk forward to the tip of the rostrum. The position of the tip of the rostrum was compared to the position of the distal end of the eye.

The length of the carpus was measured from tip to tip via the longest path. The length and width of the palm (base of the propodus) of the right chelae (present on pereiopods 1 and 2) were measured (Figure 5) and the ratio of width/length used to determine inflation of the palm. The length was measured from where the dactyl meets the palm directly





(C). The position of the tip of the rostrum was compared to the position of the distal end was measured from the palm to the tip (H), while the width was measured at the tip (not Figure 5: The morphological measurements that were taken in order to determine any changes in the pelagic group over time. The carapace height was measured near the middle of the carapace at the greatest height (A). The carapace outline is highlighted. straight back to the end of the carapace (B). The length of the rostrum was measured The carapace length was measured in the standard manner from just behind the eye path (E). The length (F) and width (G) of the palm (base of the propodus) of the right from where the dactyl meets the palm directly back to the end of the palm. The width measured, with the spread measured at the base of the tuft regardless of angle. The of the eye (D). The length of the carpus was measured from tip to tip via the longest was measured from side to side where the dactyl meets the palm. The dactyl length chelae (present on pereiopods 1 and 2) were measured. The length was measured setal length was measured from the tip to the base of the setae at the longest point. shown). The length (I) and spread (not shown) of the setae on the dactyl were



Perelopod 1 (ventral view)



Pereiopod 2 (dorsal view)

back to the end of the palm. The width was measured from side to side where the dactyl meets the palm. The dactyl length was measured from the palm to the tip, while the width was measured at the tip (Figure 5). The full length of the propodus consisted of the palm length measurement and the dactyl length measurement together. The length and spread of the setae on the dactyl were measured (Figure 5), with the spread measured at the base of the tuft regardless of angle. The setal length was measured from the tip to the base of the setae at the longest point. The length of the right carpus of pereiopods 1 and 2 was measured and compared as a ratio of the carpus length against the length of their respective chela palm (Figure 5).

All measurements were made in the same manner. The euthanized shrimp was placed onto a microscope slide. The slide was placed in a Bausch and Lomb Stereozoom 7 microscope and the animal was manipulated with dissecting probes to fully expose each part to be measured. Digital photographs were taken of each of these positions using a Nikon D70 digital camera with a Scopetronix® microscope adapter. The magnification used and the frame number were recorded for each photograph. Each measured dimension of the shrimp was calculated via the measuring tool in GIMP 2<sup>®</sup> in pixels, and converting the actual length based on a calibration table that had been prepared via stage micrometer for the microscope at each level of magnification.

#### **Statistical Analysis:**

All measurements were entered into Excel spreadsheets and the SPSS-12 statistical package. The data were cleaned by removing all outliers (data points which were more than 2 standard deviations from the mean of their group) and checked visually for normality. Statistical differences that developed between the treatments and among months were assessed by ANOVA and the Tukey post-test. In some cases; significant differences did not develop among the relevant groups but the trend of the data suggested that incipient differences may be beginning to develop. Therefore, regression and ANCOVA was also used to analyze the trends with time, comparing the two experimental groups (Sokal and Rohlf 1995, Lowry 2013). The trends were considered to be different if the ANCOVA indicated that either their regression slopes or Y-intercepts were significantly different. In all analysis, a probability of less than 0.05 was considered to indicate a significant difference. In the plots, regression lines were drawn whenever the trends of the benthic and pelagic groups were different from one another or when either or both were significantly different from zero slope. If no line was marked for a group the trend of that group was neither significantly different from the other group nor from zero.

#### **RESULTS:**

### **Overall Condition of Shrimp During the Experiment**

Although most of the shrimp remained alive and active throughout the experiment, several events and observations suggested that they may not have been living in optimal conditions. First, although this same species of shrimp living in a different tank during the time of the experiments reproduced continually, very little reproduction occurred in the experimental tanks. None of the shrimp in either of the experimental conditions (benthic or pelagic) produced viable offspring while in the tanks. Only one benthic shrimp, at the very end, was berried (carrying eggs externally) while no shrimp in the pelagic tank ever proceeded that far. Furthermore, shortly after the partial water change of March 3, 2012 (Month 1), nearly 50% of the shrimp in the pelagic tank died suddenly and one died in the benthic tank as well. The cause of this one-time die-off is not clear. At any rate, this loss of experimental subjects and their lack of reproduction under experimental conditions prevented any study of generational changes and produced a relative shortage of pelagic individuals near the end of the experiment. However, enough shrimp remained for monthly behavioral measurements from at least 10 for each benthic and pelagic group (Table 4a) and morphological measurements of a least 10 individuals for the benthic group and 8-10 individuals in the pelagic group (Table 4b).

Table 4: Number of shrimp used from each group for each set of measurements. Month 0 only had one set recorded which was used as a baseline for both the benthic and pelagic groups.

A: Number of shrimp in behavior measurements

Month	Benthic	Pelagic	
0	20		
1	10	10	
2	25	23	
3	20	20	
3 4 5 6	20	20	
5	20	20	
6	15	15	

B: Number of shrimp in morphological measurements

Month	Benthic	Pelagic
		_
0	24	
1	10	10
2	10	8
3	10	10
4	10	8
5	10	8
6	15	15

## **Swimming Behavior**

The shrimp in both experimental conditions maintained active movement and foraging behavior throughout the experiment. However, subjective observations of the animals within their experimental tanks indicated that neither group spent a majority of its time swimming. As I expected, the animals in the benthic tank crawled around the rock substrate with just a few swimming now and then. The pelagic shrimp had no such substrate to cling to, but instead of swimming, most of them clung to the few surfaces available to them such as the bottom of the pump, the intake funnel or the glass sides of the tank. In the experimental tests of swimming, the behavior varied widely with the proportion swimming at any given moment being generally around 18 percent (Figure 6). However, the pelagic group displayed a downward trend of less swimming, instead of the upward trend expected. The benthic group also had a decreasing trend.

#### **Morphological Measurements**

Measurements on the Body:

The carapace length was the shortest in month 0 as the shrimp were apparently still young. In later months, the carapace length was slightly longer (Figure 7). The only exception to this was the last few months in the pelagic group in which the only individuals left were slightly smaller than the average for the previous months (Figure 7).



Figure 6: Changes in the average proportion of shrimp which swam during the swimming test, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Error bars are 95% confidence intervals for the data. Regression suggests that a significant negative trend may be developing in the both groups, and ANCOVA indicated that this trend was significantly different between the groups.



Figure 7: Changes in carapace length (a measure of body size) of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Most shrimp in subsequent months were larger than at month 0, except for the last pelagic groups. Regression suggests that a significant positive trend may be developing in the benthic group, which has a slope significantly greater than the pelagic group. The pelagic group has no significant slope.

This artifact made the regression suggest that the benthic animals increased in size, while the pelagic animals stayed smaller (Table 5). The carapace height followed a similar pattern as the length (Figure 8). The aspect ratio (length/height) of the carapace also differed little between the benthic and pelagic (Figure 9) throughout the experiment. Rostrum length, while it varied slightly among individuals (Figure 10), did not show any consistent trend with time in either the benthic or pelagic groups. In both groups throughout the entire experiment, the rostrum projected past the eyestalks in some individuals and in others, it did not (Figure 11).

#### Measurements on Pereiopod 1:

The length of the carpus on pereiopod 1 did not change significantly with time in either group (Figure 12). The propodus length declined slightly in both groups, but regression and ANCOVA indicated that a trend may be developing with the pelagic decreasing more (Figure 13; Table 6). The length of the palm of the first propodus did not change significantly, but regression suggested a declining trend may be beginning (Figure 14, Table 6). The width of the palm of the propodus declined significantly over time in both groups (Figure 15; Table 6), and ANCOVA indicated that there was no significant difference in this declining trend between the two groups. Since the palm width declined more that the palm length did, the palm aspect ratio (length/width) increased slightly over time in the benthic group (Figure 16, Table 6). Regression and ANCOVA suggested that there was a weaker trend in the same direction in the pelagic group (Table 6). There was

Table 5: Regressions of changes in proportion swimming and body measurements during the six months of the experiment. For every regression, x=month. In the comparison section, "reg p" is the probability that the slopes of the benthic and pelagic regressions are not different. ANCOVA P is the probability that their Y intercepts are the same (valid only if the slopes are not different).

arison	COVB D	1	ł	ı	ł	i
Compe	reg p ar	0.0078	>0.001	>0.001	0.02	0.02
	p value	>0.001	0.342	0.064	0.008	>0.001
jic	P4	30	0.92	4.0	7.4	16
Regn	đf	778	11	80	67	69
	Slope	-022	-0.018	-0.039	0.023	-0.016
	Yint	0.23	2.6	2.0	<u>_</u>	0.42
	p valve	0.030	>0.001	0.003	0.94	0.20
nic	P	4.7	17	10	0.006	1.7
Bent	đ	617	8	78	11	78
	Slope	.008	0.059	0.047	-0.001	-0.005
	Yint	0.21	2.6	1.9	1.4	0.4
		Proportion Swimming	Carapace Length	Carapace Height	Carapace Aspect Ratio	Rostrum Length
Fig #		9	2	•••	0	9



Figure 8: Changes in carapace height (a measure of body size) of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Most shrimp in subsequent months were larger than at month 0, except for the last pelagic groups. Regression suggests that a significant positive trend may be developing in the benthic group, which has a slope significantly greater than the pelagic group. The pelagic group has no significant slope.


Figure 9: Changes in carapace aspect ratio (a measure of carapace inflation, a lower ratio = more inflation) of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No major changes in carapace inflation developed in either group. Regression suggests that a significant positive trend (toward less inflation) may be developing in the pelagic group, which also has a slope significantly greater than the benthic group. The benthic group has no significant slope.



Figure 10: Changes in rostrum length of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). There was no systematic change in rostrum length in either group over the course of the experiment. Regression suggests that the pelagic group may be trending toward a shorter rostrum. The pelagic group has a significant negative slope, which is significantly less than that of the benthic group. The slope of the benthic group is not significant, suggesting that no change is developing in rostrum length in that group.



Figure 11: Changes in the proportion of individuals from the benthic and pelagic groups in which the rostrum projected past the eye, by month. There were no systematic changes in either group, though regression suggests that the proportion of individuals with a rostrum longer than the eye may be decreasing in the pelagic group.



Figure 12: Changes in the carpus length on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). There was no systematic change in carpus length in either group over the course of the experiment. Regression also failed to find any significant trends developing.



Figure 13: Changes in the propodus length on pereiopod 1 for individuals in the benthic and pelagic groups, HSD post-test). The pelagic group declined significantly. While the benthic group did not decline significantly, regression suggests a declining trend in both groups. ANCOVA indicates the decline is greater in theby month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey pelagic group.

Table 6: Regressions of changes in first pereopod measurements during the six months of the experiment. For every regression, x=month. In the comparison section, "reg p" is the probability that the slopes of the benthic and pelagic regressions are not different. ANCOVA P is the probability that their Y intercepts are the same (valid only if the slopes are not different).

				Benthic					Pelagic-			Comp	arison
Fic#				Regress	ion				Regressi	00			
•		Y int	Slope	ġ	H	p value	Y int	Slope	đť	H	p value	reg p	ADCOVA D
12	Carpus Length	0.36	0.005	78	15	0.22	0.36	•	73	0.002	0.97	0.93	23
m	Propodus Length	0.59	-0.014	2	8.9	0.004	0.59	-0.023				0.098	0.042
14	Palm Length	0.32	-0.009	11	5.8	0.018	0.31	-0.017				0.11	>0.001
51	Palm Width	0.39	-0.03	11	66	>0.001	0.37	-0.03				16.0	0.13
91	Palm Aspect Ratio	0.86	0.062	26	41	>0.001	0.88	0.031	2	15		0.016	1
11	Carpus Length/Propodus Length	0.62	0.027	80	1.1	0.010	0.61	0.032				0.77	0.84
8	Dactyl Length	0.26	-0.004	1	2.6	0.11	0.28	-0.006				0.61	0.096
61	Dactyl Width	0.082	-0.003	2	6.9	0.011	0.081	-0.002				0.7	0.95
ន	Dactyl Length/Palm Length	0.87	0.007	2	0.22	0.64	96.0	0.041				0.13	>0.001
31	Setae Length	0.28	-0.004	26	1.6	0.20	0.27	0.001				0.25	0.22
8	Setae Spread	0.074	-0.002	8	8.9	0.004	0.077	-0.0				0.26	0.95
8	Setae Length/Spread	4.0	0.035	26	0.24	0.63	3.8	0.214				0.062	0.38
처	Setae Length/Dactyl Length	Ξ	0.001	2	0.001	0.98	6.0	0.034				0.15	0.88
35	Setae Length/ Palm Length	0.931	0.013	22	0.38	0.54	0.95	0.068				0.48	>0.001

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Figure 14: Changes in the length of the palm on the propodus of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each o ANOVA with Tukey HSD post-test). There was no systematic change in palm length in either group over ther (the course of the experiment. Regression, however, suggests that a significant trend toward shorter palm length may be developing in both groups, and ANCOVA suggests that this trend may be more pronounced in the pelagic group.



Figure 15: Changes in the width of the palm on the propodus of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Both groups developed significantly narrower palms during the course of the experiment, but were not different from each other. Regression also suggests a significant trend toward decrease in palm width in both groups, but ANCOVA indicates that this trend was not significantly different between the groups.



Figure 16: Changes in the palm aspect ratio (an indicator of inflation of the palm) on the propodus of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). The palm aspect ratio of the benthic group became significantly larger (less inflated) in the benthic group but not in the pelagic group. Regression suggests a trend toward higher aspect ratio (less inflation) in both groups, and ANCOVA indicates that this trend is significantly more pronounced in the benthic group.

no significant change in the ratio of carpus length to propodus length in either group (Figure 17), though regression suggested that a trend toward a longer carpus in relation to the propodus was developing to about the same extent in both groups (Figure 17, Table 6). Neither dactyl length nor width changed significantly for either group (Figures 18, 19), though regression and ANCOVA indicated that the pelagic group may be trending toward a shorter dactyl and both groups may be trending equally toward a narrower dactyl. There was also no consistent change in the ratio between the lengths of the palm and the dactyl (Figure 20). Regression and ANCOVA suggest that the pelagic group but not the benthic group may be developing a trend toward a greater ratio of dactyl length to palm length (Table 6). The length and spread of the tuft of setae on dactyl 1 also did not change over time for either group (Figures 21, 22), although regression and ANCOVA suggested an equal trend toward narrowing of the setal tuft in both groups and toward an increased aspect ratio of the setal tuft in the pelagic group (Figures 21-23, Table 6). The ratio of the length of the setae to the length of the dactyl or to the length of the palm did not change with time on pereiopod 1 for either group (Figures 24, 25), although regression and ANCOVA suggest that a trend toward longer setae as compared to the palm may be developing in the pelagic (but not the benthic) group (Figure 25, Table 6).

Measurements on Pereiopod 2:

The length of the carpus on pereiopod 2 did not change significantly with time in either group (Figure 26, Table 7). The length of the propodus declined slightly in both groups



Figure 17: Changes in the ratio of carpus length to propodus length on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences between the two groups developed over time. Regression, however, suggests a trend toward an increase in the ratio in both groups. ANCOVA indicates this trend is not significantly different between the groups.



Figure 18: Changes in the length of the dactyl of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in dactyl length during the experiment. Regression suggested that the pelagic group may be trending toward decreased dactyl length, but ANCOVA indicates that the trend is not significantly different from that of the benthic group. There is no significant trend in the benthic group.



Figure 19: Changes in the width of the dactyl of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in dactyl length during the experiment. Regression suggested that both groups may be trending toward a narrower dactyl. ANCOVA indicated that there was no significant difference in this trend between the two groups.



Figure 20: Changes in the ratio of dactyl length to palm length on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in this ratio during the experiment. Regression suggested that the pelagic group may be trending toward greater dactyl length in proportion to palm length. The regression was not significant for the benthic group. ANCOVA indicated that the trend toward a higher ratio was greater in the pelagic group than in the benthic group.



Figure 21: Changes in the length of setae on the dactyl of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Neither group changed significantly in setal length during the experiment, and regression found no trends developing.



Figure 22: Changes in the spread of setae on the dactyl of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Neither group changed significantly in setal spread during the experiment. However, regression and ANCOVA suggest that both groups may be trending equally toward a narrower setal spread.



Figure 23: Changes in the aspect ratio (length/spread) of setae on the dactyl of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Neither group changed significantly in setal tuft aspect ratio during the experiment. However, regression and ANCOVA suggest that the pelagic group may be increasing in aspect ratio. Although the benthic group has no such trend, the regression slopes of the two groups are not significantly different from one another.



Figure 24: Changes in the length of the setal tuft as compared to the length of the dactyl on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Neither group changed significantly in the ratio during the experiment, nor did regression suggest any trends developing.



Figure 25: Changes in the length of the setal tuft as compared to the length of the palm of the propodus on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in the ratio during the experiment. However, regression and ANCOVA suggested that a trend of longer setae in comparison to the palm may be developing in the pelagic group but not the benthic group.



Figure 26: Changes in the length of the carpus of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in carpus length during the experiment, nor did regression suggest any trends developing.

regression, x=month. In the comparison section, "reg p" is the probability that the slopes of the benthic and pelagic regressions are not different. ANCOVA P is the probability that their Y intercepts are the same (valid only if the slopes are not different). Table 7: Regressions of changes in second pereopod measurements during the six months of the experiment. For every

				-Benthic					-Pelagic-			-Compa	rison
Fig #				-Remea	tion-				Repressi	01		•	
0		Y int	Slope	, H	54	p valve	Y int	Slope	ţ,	H	p value	regp	incova p
8	Carpus Length	0.67	0.000	11	3.2		0.66		11	0.093	0.762	1.20	2.50
12	Propodus Length	0.62	-0.019	11	8		0.63		89	51	>0.001	0.096	0.26
*	Palm Length	0.32	-0.014	22	11		0.32		1	74	>0.001	0.06	×0.001
2	Palm Width	0.36	-0.022		청		0.32		2	50	>0.001	0.82	0.01
8	Palm Aspect Ratio	6.0	0.016		2.4		1.0		89	3.1	0.082	0.02	,
	Carpus Length/Propodus Length	Ξ	0.062		16		Ξ		<u></u>	31	>0.001	0.00	0.55
32	Dactyl Length	0.29	-0.005	2	2.5	0.12	0.31	-0.006	66	3.8	0.5	0.77	8.0
8	Dactyl Width	0.079	-0.002		3.8		0.075		69	9.4	0.003	0.48	0.03
*	Dactyl Length/Palm Length	0.93	0.04		3.9		0.97		89	2	>0.001	0.067	0.0036
8	Setae Length	0.32	-0.012		53		0.31		89	12	0.20	0.01	1
8	Setae Spread	0.079	-0.002		3.8		0.072		80	6.0	0.017	0.36	0.36
31	Setae Length/Spread	4.8	-0.009		0.007		4.4		2	6.1	0.017	0.0	0.20
8	Setae Length/Dactyl Length	Ξ	-0.009		0.17		1.0		2	8.1	0.006	0.06	0.63
8	Setae Length/Palm Length	1.0	0.022		0.70		0.95		67	2	>0.001	0.00	,

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(Figure 27, Table 7), and the decline was about equal in both groups. The length and width of the palm of the propodus declined over time in both groups (Figures 28, 29), with the pelagic length declining faster than the benthic length (Table 7). No significant change in palm aspect ratio (length/width, a low value indicates an inflated palm) occurred, though the pelagic group may possibly have been trending toward a more inflated palm than the benthic group (Figure 30, Table 7). Since the propodus length declined slightly, but carpus length did not, the ratio of carpus length/propodus length also increased slightly over time for the benthic group (Figure 31). Regression suggested that the pelagic group was trending in the same direction. This increase, however, was not significantly different between the benthic and pelagic (Table 7). Dactyl length and width did not change significantly for either group (Figures 32, 33), although regression suggested that there may have been a negative trend in the pelagic group (Table 7). There was also no consistent change in the ratio between the length of the dactyl to the length of the palm (Figure 34). Although again, regression suggests a greater positive trend in the pelagic group than in the benthic group (Table 7). The length of the tuft of setae on dactyl 2 decreased slightly for the benthic, while remaining similar for the pelagic (Figure 35). The spread of the setae did not change significantly, though both groups may have been on a downward trend (Figure 36). The aspect ratio (length/spread) of the tuft of setae also did not change significantly, though regression indicates a possible trend developing toward increase in aspect ratio of the pelagic group (Figure 37, Table 7). The ratio of the length of the setae to the length of the dactyl did not change with time on pereiopod 2 (Figure 38), though regression suggested the pelagic group may be



Figure 27: Changes in the length of the propodus of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Both groups declined about equally in propodus length during the course of the experiment.



Figure 28: Changes in the length of the palm of the propodus of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Both groups had a significant decline in palm length. ANCOVA indicated that the declining trend in the pelagic group was greater than that in the benthic group.



Figure 29: Changes in the width of the palm of the propodus of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Both groups had a significant decline in palm width. ANCOVA indicated that the declining trend in the pelagic group was greater than that in the benthic group.



Figure 30: Changes in the aspect ratio (length/width, a low value indicates an inflated palm) of the palm of the propodus of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences in aspect ratio developed during the experiment. However, regression and ANCOVA suggested that while the trend of neither group was significantly different from zero, the two groups were significantly different from each other, with the pelagic group trending more clearly downward than did the benthic group.



Figure 31: Changes in the ratio of carpus length to propodus length of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). The benthic group increased significantly in this ratio. Regression suggests that the pelagic group was tending in the same direction, and ANCOVA indicates that the trends of the two groups were not significantly different from each other.



Figure 32: Changes in dactyl length of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences in dactyl length developed during the experiment, nor did regression indicate any significant trends.



Figure 33: Changes in dactyl width of pereiopod 2 for individuals in the benthic and pelagic groups, by month. No significant differences in dactyl width developed during the experiment. However, regression suggested that the pelagic group may have been declining in dactyl width, and ANCOVA indicated that this declining trend was greater than that in the benthic group.



Figure 34: Changes in the ratio of dactyl length to length of the palm of the propodus on pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences developed over time in either group. However, regression suggests that this ratio may have been increasing in the pelagic group, and ANCOVA indicates the increasing trend in the pelagic group was greater than that in the benthic group.



Figure 35: Changes in the length of the setal tuft on the dactyl of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences developed over time in either group. However, regression suggested that a declining trend was developing in the benthic group while not in the pelagic. ANCOVA indicated that the two groups were trending in significantly different directions.



Figure 36: Changes in the spread of the setal tuft on the dactyl of pereiopod 2 for individuals in the benthic and pelagic groups, by month. No significant differences developed over time in either group. However, regression suggested that a declining trend was developing in both groups. ANCOVA indicated that the decline was not significantly different between the two groups.



Figure 37: Changes in the aspect ratio (length/spread) of the setal tuft on the dactyl of pereiopod 2 for individuals in the benthic and pelagic groups, by month. No significant differences developed over time in either group. However, regression suggested that an increase in aspect ratio may be developing in the pelagic group but not in the benthic. ANCOVA indicated that the two groups were not trending in significantly different directions.



Figure 38: Changes in the length of the setal tuft as compared to the length of the dactyl on pereiopod 2 for individuals in the benthic and pelagic groups, by month. No significant differences developed over time in either group. However, regression suggested that an increase in the setal length/dactyl length may be developing in the pelagic group but not in the benthic. ANCOVA indicated that the two groups were not trending in significantly different directions.

increasing (Table 7). Similarly, the length of the setal tuft compared to the length of the palm increased significantly in the pelagic group while it did not in the benthic group (Figure 39, Table 7).



Figure 39: Changes in the length of the setal tuft as compared to the length of the palm of the propodus on pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). There was a significant increase in this ratio for the pelagic but not for the benthic group. Also, regression indicated an increasing trend in the pelagic group but not in the benthic group, and ANCOVA indicated that the trends of the two groups were significantly different.
## **DISCUSSION:**

Although much variability was observed in these shrimp over the six months of this experiment, the variability was mostly not in the pattern that would be expected if the pelagic group was transitioning to the morphology seen in *H. palahemo*. Few significant long term differences developed between the benthic and pelagic groups and even fewer of these were in the direction that would be expected if the two groups were diverging. If the pelagic group were becoming more like *H. palahemo*, for example, the carapace would become more inflated, the rostrum would shorten, the chelae would become less inflated, and the carpus would become longer than the chelae (Tables 1, 2). However, there was no sign of the carapace aspect ratio and rostrum differentiating between the benthic and pelagic groups (Figures 9, 10). Since the last few samples of the pelagic group were smaller than average as measured by carapace length (Figure 7), it is possible that this size differential might obscure developing differences in other features if there is a relationship between size and the development of those features. To test for this, each of the features predicted to change in the pelagic group was examined (Table 2) to see if there was a significant change in the feature with size (carapace length) (see appendix 1). If there was a relationship, regression was used to adjust all the measurements of that feature to what they would have been on a shrimp of average carapace length (2.5 mm). This size-adjusted data was retested to see if any of the relationships predicted in Table 1 and 2 appeared. After this adjustment, there was still no evidence that the carapace of the pelagic group was becoming more inflated (Figure 40). If anything, after the carapace aspect ratio was corrected for size, the adjusted pelagic group appeared to be beginning to



Figure 40: Changes in the adjusted (normalized to carapace length 2.5) carapace aspect ratio (a measure of carapace inflation, a lower ratio = more inflation) of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No major changes in carapace inflation developed in either group. Regression suggests that a significant positive trend (toward less inflation) may be developing in the pelagic group, which also has a slope significantly greater than the benthic group. The benthic group has no significant slope.

form a trend in the opposite direction as expected and becoming less inflated, as demonstrated by the significant positive regression for the pelagic group in Figure 40. Similarly, when adjusted the rostrum length does have a declining trend, but not significantly more than that of the benthic group which also declines (Figure 41). Both groups also had a similar proportion of individuals in which the rostrum projected past the eyestalks (Figure 11), though the negative regression suggests the pelagic group may be declining in this feature (Figures 10, 41), a trend which was predicted for this group (Table 2).

On the first pereiopod, there were a few indications that a trend may be developing toward a change between the benthic and pelagic groups. In the pelagic group, the palm aspect ratio did have an increasing trend as predicted (Table 2), but not at a rate faster than the benthic group was increasing (Figures 16, 42). The length of the carpus/length of the propodus also trended upward both in the benthic and in the pelagic groups, but not at significantly different rates (Figure 17). Several features associated with the dactyl and setae, which are used to scrape algae off the rocks if benthic, or sweep algae from the water if pelagic, also trended differently between the groups. The setal tuft, which is attached to the dactyl, was expected to become longer and perhaps more robust in the pelagic group, but to remain shorter in the benthic group because it is continually scraped against the rocks. When adjusted for the size of shrimp, the length of dactyl/length of palm of the pelagic group seemed to be growing a proportionally longer dactyl than the benthic as expected, as shown via regression (Figure 43, Table 8). The setal



Figure 41: Changes in the adjusted (normalized to carapace length 2.5) rostrum length of shrimp from the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). There was no systematic change in rostrum length in either group over the course of the experiment. Regression suggests that both groups may be trending toward a shorter rostrum. However, ANCOVA indicates that the trend of both groups are not significantly different from each other.



Figure 42: Changes in the adjusted (normalized to carapace length 2.5) palm aspect ratio (an indicator of inflation of the palm) on the propodus of pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Regression suggests a trend toward higher aspect ratio (less inflation) in both groups, however, neither is significantly different than the other.



Figure 43: Changes in the ratio of the adjusted (normalized to carapace length 2.5) dactyl length to palm length on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in this ratio during the experiment. Regression suggested that the pelagic group may be trending toward greater dactyl length in proportion to palm length. The regression was not significant for the benthic group. ANCOVA indicated that the trend toward a higher ratio was greater in the pelagic group than in the benthic group.

experiment. For every regression, x=month. In the comparison section, "reg p" is the probability that the slopes of the benthic and pelagic regressions are not different. ANCOVA P is the probability that their Y intercepts are the same (valid only if the slopes are Table 8: Regressions of changes in adjusted measurements (corrected for body size 2.5 mm) during the six months of the not different).

				-Benthic					-Pelagic				arison
Hig #				-Regress	IOI				-Regress				
		Y int	Slope	đ	H	p value	Y int	Slone	df	H	p value	TEE D	
우	Carapace Aspect Ratio	1.4	0.006	11	0.80	0.37	n	0.21	67	7.0	0.01	0.13	
41	Rostrum Length	0.40	-0.009	76	7.0	0.01	0.41	-0.016	68	11	×0.001	0.19	0.952
4	Palm Aspect Ratio	0.84	0.054	2	30	>0.001	0.86	0.035	<u></u>	18	>0.001	0.15	0.24
4	Dactyl Length/Palm Length	0.85	0.014	8	0.69	0.41	<b>56</b> .0	0.041	61	1.1	0.007	0.23	>0.001
4	Setae Length/Palm Length Pereopod 2:	16.0	0.018	11	0.76	<u>039</u>	<b>56</b> .0	0.067	8	=	0.001	0.097	0.013
<del>5</del>	Carpus Length/Propodus Length	=	0.043	2	6.8	0.011	Ξ	0.066	85	8	>0.001	0.27	0.087
\$	Dactyl Length/Palm Length	0.94	0.048	80	5.0	0.029	1.0	0.082	8	16	>0.001	0.25	0.0094
4	Setae Length/Dactyl Length	Ξ	-0.025	6	Ξ	0.30	1.0	0.041	8	6.7	0.012	0.041	1
8	Setae Length/Palm Length	1.0	0.017	67	0.36	0.55	0.98	0.14	28	38	>0.001	0.001	,

length/spread was also increasing in the pelagic, but not at a significantly different rate from the benthic group (Figure 23). Also, this changing ratio between length and spread of the setal tuft was caused mainly by a narrowing rather than a lengthening of the tuft (Figures 21 to 23, Table 6), a trend which may be associated with a pelagic lifestyle but was not expected. The setal length/dactyl length ratio did not show an increasing trend in either group (Figure 24). Finally, the pelagic setal length as compared to the length of the palm trended toward an increase in this ratio (Figure 25), a relationship which remained even after adjusting for animal size (Figure 44). This change was primarily due to a shortening of the palm rather than to a lengthening of the setae (Figures 14, 21, Table 6). The pereiopods themselves did not appear to be taking on the same morphology as shown in Fig. 11 of Kensley and Williams (1986).

The second pereiopod showed some similar trends as the first and some different trends. Although no consistent significant differences developed between the benthic and pelagic groups, the aspect ratio of the palm in the pelagic group had a significant negative trend by regression, a result opposite of what was expected (Figure 30, Table 7). The palm aspect ratio of the benthic group did not increase significantly, but the trend was significantly more positive than that of the pelagic group (Figure 30, Table 7). This was also an unexpected result. While the length of the carpus compared to the length of the propodus did trend toward an increase as expected in the pelagic shrimp, it also did so in the benthic shrimp and there was no significant difference between the two trends of the groups (Figure 31, Table 7). This relationship remained the same after correcting for



Figure 44: Changes in the adjusted (normalized to carapace length 2.5) length of the setal tuft as compared to the length of the palm of the propodus on pereiopod 1 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Neither group changed significantly in the ratio during the experiment. However, regression and ANCOVA suggested that a trend of longer setae in comparison to the palm may be developing in the pelagic group but not the benthic group. The benthic slope is not significant.

body size (Figure 45, Table 8). The length of the dactyl compared to the length of the palm had a significantly increasing trend in the pelagic group while the benthic did not (Figure 34, Table 7). This relationship, which was expected based on the expectation that the setal tuft and associated structures would become larger in the pelagic group, persisted after adjustment for animal size (Figure 46, Table 8). As on pereiopod 1, the aspect ratio of the setal tuft trended toward a significant increase in the pelagic group while it did not in the benthic group, though the trends of the pelagic and benthic groups were not significantly different (Figure 37). This trend was primarily due to a narrowing rather than to a lengthening of the setal tuft (Figures 35, 36, Table 7), as was also seen on pereiopod 1. The length of the setae compared to the length of the dactyl and of the palm followed a significant upward trend with time as expected (Figures 38, 39, Table 7), while they did not in the benthic group. This relationship also persisted after adjusting for animal size (Figures 47, 48, Table 8). The pereiopods themselves did not appear to have the same morphology as shown in Fig. 11 of Kensley and Williams (1986).

In parallel with the morphological results, there was substantial variability in the proportion of time spent swimming by both groups during the course of the experiment (Figure 6). The pelagic group was expected to gradually increase the amount of time they voluntarily spent swimming in the water column, however, there was a significant trend toward a decrease in swimming among the pelagic shrimp, more so than seen in the benthic group (Figure 6). It is unclear why this would occur.



Figure 45: Changes in the adjusted (normalized to carapace length 2.5) ratio of carpus length to propodus length of pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). Regression suggests that both groups were tending in the same upwards direction, and ANCOVA indicates that the trends of the two groups were not significantly different from each other.



Figure 46: Changes in the adjusted (normalized to carapace length 2.5) ratio of dactyl length to length of the palm of the propodus on pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences developed over time in either group. However, regression suggests that this ratio may have been increasing in the both groups, and ANCOVA indicates the increasing trend in the pelagic group was greater than that in the benthic group.



Figure 47: Changes in the adjusted (normalized to carapace length 2.5) length of the setal tuft as compared to the length of the dactyl on pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). No significant differences developed over time in either group. However, regression suggested that an increase in the setal length/dactyl length may be developing in the pelagic group but not in the benthic. ANCOVA indicated that the two groups were trending in significantly different directions.



Figure 48: Changes in the length of the adjusted (normalized to carapace length 2.5) setal tuft as compared to the length of the palm of the propodus on pereiopod 2 for individuals in the benthic and pelagic groups, by month. Different letters indicate groups which were significantly different from each other (ANOVA with Tukey HSD post-test). There was a significant increase in this ratio for the pelagic but not for the benthic group. Also, regression indicated an increasing trend in the pelagic group but not in the benthic group, and ANCOVA indicated that the trends of the two groups were significantly different.

These results, taken together, do not strongly support the hypothesis that the distinctive characteristics seen in *H. palahemo* are simply morphological adjustments that could occur in *H. rubra* when placed in a pelagic environment. No consistent, significant morphological differences developed between the two groups during the course of the experiment. Several significant trends did seem to be developing in the expected direction in the pelagic group, such as a slightly shortening rostrum and an increase in the aspect ratio and length of the feeding setae as compared to the length of other leg parts on both pereiopods. A number of other expected differences, however, such as the inflation of the carapace and chelae, a lengthening of the carpus, and an increase in time spent swimming had ambiguous results or even appeared to be trending in the opposite direction from what was expected. While they do not provide strong support for the hypothesis, however, they also do not negate it. This experiment may not have provided the optimized set of strongly contrasting conditions needed to fully test the hypothesis. As indicated in the results, the shrimp did not appear to be living in optimal conditions, which may have inhibited their growth and altered their behavior. For example, even though steps were taken to reduce the current in the tanks, including splitting the outflow lines and reducing the suction at the pump intake by using a funnel and screen, these steps may not have produced a slow enough current for the shrimp. Further, though plenty of benthic substrate was provided for the benthic group and substrate was minimized for the pelagic group, a substantial portion of the pelagic shrimp persistently clung to the few surfaces available to them rather than swimming freely in the water.

There are a few other factors that might have influenced the environment to disturb the shrimp as well. Upon receiving the shrimp, the salinity of their water was 14 ppt and it was adjusted slightly upward to approximately 16-18 ppt, which is closer to the middle of the recommended range in the literature instead of the lower end (Hallweg, 2008). During the experiment, salinity remained in this range, or occasionally drifted as high as 26 ppt. Yet these shrimp showed very little reproduction during the experiment, preventing any study of generational changes in morphology. This result mirrored that of the supplier (opaeula.com), who reported that they were having trouble getting the shrimp to reproduce in captivity (Cowles personal communication). Meanwhile, a separate tank of the same species was maintained at approximately 23 ppt with little stirring during the time of the experiment, and the individuals in that tank reproduced abundantly. It is also possible that the food provided was not the preferred food choice and they did not receive the normal nutrition. The powdered *Spirulina* algae provided for the pelagic group stayed mostly suspended, but substantial portions settled to the bottom. Perhaps adding less food and/or switching to a live, motile alga may help keep the food fully suspended in the pelagic tank. The shrimp also may have been disturbed by actions that maintained the experimental conditions. The benthic tank needed to have bottom sediment periodically removed while the pelagic tank was scrubbed to prevent algal growth on surfaces. Both of these actions may have disturbed the shrimp. Hiding places in the tank were also limited. This species generally likes to hide in crevices or at least have the security of having hiding spots available. If the species has a strong tendency to seek out and alight on solid surfaces, as suggested in this experiment, it may be very difficult to

create an experimental chamber which models the conditions found in the deep pool where the pelagic *H. palahemo* lives (Figure 2 inset), in which most of the benthic substrate is presumably so deep below the water that the shrimp are forced to remain pelagic to remain near their food. Hardly any chamber small enough to fit in a lab would have that characteristic. Perhaps a planktonkreisel with gentle flow would both solve the problem of settling algae and discourage the shrimp from attaching to the bottom as well.

Other reasons for an incomplete divergence in the two experimental groups could be that six months may have been too short a period for these changes to manifest themselves in the G0 population. Were the experiment run longer, more clear changes may have occurred in the populations. Furthermore, changes may likely have been more pronounced if another generation or two were included in the data. Changes between the *H. rubra* and *H. palahemo* form may be epigenetic and only fully manifest themselves between generations. As these shrimp did not reproduce, no conclusive evidence of any inter-generational changes was possible.

The possibility still remains that the reason that divergence could not be shown between the benthic and pelagic groups is that the benthic *H. rubra* and the pelagic *H. palahemo* truly are actually separate species and not just different morphologies induced by different feeding modes and lifestyles. Further testing of this hypothesis will need to be done. The design of this experiment would not be able to fully reject this hypothesis. DNA sequencing would be an excellent compliment to this study and may provide a more complete answer to the question at hand.

In summary, while this experiment provided some fascinating hints that *H. rubra* might convert to the morphology of *H. palahemo* when placed under pelagic conditions and that *H. palahemo* might therefore just be another form of *H. rubra*, it did not conclusively support or refute that hypothesis. The experiment did, however, suggest modified conditions that will need to be implemented to further test the hypothesis.

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	Together	Regression	Carapace Aspect Ratio	Rostrum Length Pereopod 1:	Palm Aspect Ratio	Carpus Length/Propodus Length	Dactyl Length/Palm Length	Setae Length/Spread	Setae Length/Dactyl Length	Setae Length/Palm Length Dereaned 2:	Palm Aspect Ratio	Carpus Length/Propodus Length	Dactyl Length/Palm Length	Setae Length/Spread	Setae Length/Dactyl Length	Setae Length/Palm Length