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Influence of temperature on production of the amphipod *Parhyale hawaiensis*

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Abstract

The amphipod *Parhyale hawaiensis* is a tropical species and of interest for use as a live feed in warm water marine aquaculture. Prior to the establishment of large scale culture optimal culture conditions need to be determined. The effects of temperature (20 °C, 23 °C, 26 °C, and 29 °C) on juvenile growth, survival, and generation time of the marine amphipod *P. hawaiensis* were assessed in this study. Growth was followed for 12 weeks, survival for 16 weeks and hatchling-to-hatchling generation time for 20 weeks. During juvenile production data concerning precopula behavior and mating pair productivity were obtained. Higher growth (length, weight) was seen at 26 °C (4.4 ± 0.58 mm, 2.8 ± 1.4 mg) and 29 °C (4.6 ± 0.8 mm, 2.3 ± 0.89 mg) and higher survival at 23 °C (25.2 ± 12.2%) and 26 °C (31.9 ± 3.2%). The hatchling-to-hatchling generation time at 26 °C and 29 °C was 16 weeks but was not determined at lower temperatures, as no hatchlings were observed by 20 weeks. Mating pairs were formed within two days and the productivity rate of mating pairs was 1.32 ± 0.31 juveniles per pair, at ambient room temperature (~21 °C). The data suggests culturing *P. hawaiensis* at 26 °C would enhance production. This study provides valuable data that may be used to establish large-scale production of this species.

Keywords Aquaculture, Crustacean, *Parhyale hawaiensis*, Generation time, Reproduction

Introduction

Live feed is commonly used in aquaculture for rearing the early stages of fish and invertebrates [1]. Both the ornamental and food fish aquaculture industries primarily rely on the use of artemia, rotifers, or copepods as live feeds due to their ease of culture [2]. Although these organisms are high in protein they lack key nutrients, such as polyunsaturated acids (PUFAs), and therefore they must be enriched prior to feeding. Unlike artemia and rotifers, amphipods are a natural dietary component of aquatic species, such as sportfish, *Salmo trutta* [3], and seahorses, *Hippocampus erectus* and *H. abdominalis* [4, 5]. Amphipods have successfully been used as live and frozen feed for *Poecilia reticulata*, *H. erectus*, and *Octopus maya* [6–8].

In contrast to live feeds currently used in aquaculture, amphipods contain the PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are critical nutrients for early larval development and are also high in protein [2, 9–11]. As protein is the most expensive element of feed costs, the incorporation of amphipod meal to replace fish meal has been explored [11–13], providing another incentive to pursue large-scale production of amphipods.

The amphipod, *Parhyale hawaiensis*, is a small malacostracan crustacean with circumtropical distribution [14]. This species is found globally among mangroves and macroalgae in the intertidal zone, and locally along the muddy bottoms of Florida's Indian River Lagoon [14, 15]. Large numbers of this amphipod have been found in

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Ulva lactuca culture tanks that are part of the land-based integrated multi-trophic aquaculture (IMTA) system located at Florida Atlantic University's Harbor Branch Oceanographic Institute (FAU-HBOI) aquaculture facility, as well as in the IMTA system bioreactor tanks. Although *P. hawaiensis* has been used as a model organism for molecular, genetic, and developmental research [16, 17], no definitive culture techniques have been established nor have large-scale studies been conducted to determine optimal rearing conditions. This species is a promising aquaculture candidate due to its ability to breed year-round and its high tolerance to environmental variations in both temperature and salinity [16, 18, 19]. Even in species that tolerate a wide range of conditions determining optimal conditions for growth, survival, and reproduction is a necessary step for efficient large-scale aquaculture production.

To our knowledge, no studies have been conducted regarding the influence of temperature on the growth and survival of *P. hawaiensis*. Length is typically used as a measure of growth. However, methods for measuring length have not been standardized for amphipods, with various studies reporting growth in terms of cephalothorax length, metasomatic length, or total body length [20–24]. Weight comparisons for *P. hawaiensis* have only recently been reported, with Vargas-Abúndez et al. [23] finding a significant relationship between wet weight and total body length.

A few studies have examined the reproductive success and generation time of *P. hawaiensis.* This species has been reported to become sexually mature in as little as six weeks at temperatures ranging between 18 and 25 °C [25, 26], and as long as three months when maintained at 24 °C [27]. Mature adults range in total body length from 5 to 12 mm and are sexually dimorphic making it easy to distinguish males from females [23, 28]. Reproductively mature adults exhibit precopulatory behavior prior to mating. The male grasps the female and they remain together for a few hours to days until the female molts, at which time reproduction occurs [29]. Mature females have been reported to breed every two to three weeks, producing from one to 25 embryos per brood [16]. Reported generation times vary greatly from as little as 7–8 weeks when maintained in the laboratory at 26 °C, to as long as 3.2 to 5.4 months in natural populations [16, 20]. Commercial production of this species would obviously be enhanced by determining culture conditions that would result in higher growth, faster maturity and a shorter generation time. Based on the above-mentioned information, the aim of the present study was to determine the most favorable temperature for growth, survival, and hatchling-to-hatchling generation time of *P. hawaiensis*.

Materials and methods

Culture establishment

Adult *Parhyale hawaiensis* were collected from the bioreactor tank located within the Integrated Multi-Trophic Aquaculture (IMTA) system at FAU-HBOI on June 5, 2017, using 500 µm sieves to presort for adult size. Adults were identified based on body length (> 5 mm) and secondary sexual characteristics, which are displayed as enlarged gnathopods in males and ventral brood pouches in females [16].

Twelve 4-L cylindrical plastic containers (21 × 15.2 cm; H x W) equipped with air stones were filled with 2-L of UV sterilized filtered saltwater from the FAU-HBOI saltwater well (30–32 ppt) and held at ambient temperature (20–21 °C). Each container was stocked with 12 female and 6 male sexually mature adults (18/container, $N = 216$ total) to mimic the 2:1 female to male sex ratio found in natural populations [20]. Biofloc (25 ml) collected from the IMTA ex-situ bioreactor was added to each container every other day to serve as a food source. A 10% water exchange was conducted on alternate days. Observations of precopula behavior, adult survival and juvenile production were recorded daily for a period of 18 days, until 1200 juveniles (100/replicate) were collected to conduct temperature studies. Mating productivity was evaluated for days 12–18 and defined as the number of juveniles produced per couple.

Temperature study

The study was conducted from June to October 2017. The four experimental treatment groups consisted of water temperatures maintained at 20 ± 0.3 °C, 23 ± 0.8 °C, 26 ± 0.3 °C, and 29 ± 0.5 °C ($N = 3$ replicates/treatment). Twelve 4-L plastic containers, described above, were filled with 2-L of UV sterilized filtered saltwater and supplied with air stones to maintain desired oxygen levels, and covered with loose fitting lids. Temperatures were maintained by placing containers in rectangular trays (39 × 28 × 11 cm) filled with fresh water and equipped with aquarium heaters. A 25% water exchange with UV filtered saltwater was performed weekly with pre-heated water and freshwater was added to containers as needed to replace water lost through evaporation and maintain salinity. Each experimental container ($N = 12$) was stocked with 100 hatchlings obtained from the broodstock containers ($N = 1200$ total). The mean initial hatchling weight was 0.02 ± 0.00 mg and mean initial hatchling length was 1.38 ± 0.09 mm. Amphipods were fed daily with 10 ml of biofloc (dry weight 0.105 g/L) which had been collected from the IMTA ex-situ bioreactor and passed through a 300 µm sieve to eliminate the reintroduction of amphipods. The amount fed was calculated based on a previously conducted amphipod study (unpublished data) that used biofloc from the ex-situ bioreactor as a sole food source.

The study was interrupted in week 12 by Hurricane Irma. Survival was assessed and samples were collected to measure growth, but no hatchlings had yet been observed. As no generation time had been determined amphipod cultures were maintained, however heaters were unplugged and containers fed a two day ration as it was unclear how long the facility would be closed. Facility closure lasted for one week, during which time amphipods were not fed and temperatures in all containers reverted to ambient room temperature. Upon return, containers were checked for hatchlings but survival was not assessed; no hatchlings were observed. Heaters were added and containers returned to previous experimental temperatures and hatchling to hatchling generation time observations were resumed. Due to the low number of amphipods remaining in the containers, amphipod were not collected for weight and length measurements.

At 16 weeks, hatchlings were observed in two of the treatment groups and survival was assessed again. As amphipod density varied between containers in the remaining treatment groups (20 and 23 °C) amphipods from replicate containers were pooled and evenly redistributed between two replicate containers to obtain an adequate number (~10) of amphipods necessary for reproduction to occur. Although attempts were made to ensure equal numbers of males and females, the sex of some could not be distinguished. Hatchling observations continued for another four weeks but were halted at week 20 as no additional hatchlings were observed.

Water quality

Temperature, dissolved oxygen (YSI Pro 20; Yellow Springs, OH), pH (YSI pH100A; Yellow Springs, OH) and salinity (Fisher brand refractometer, Pittsburg, PA) were monitored daily. Total ammonia nitrogen (TAN) and nitrite (NO_2) concentrations were tested weekly (Hach colorimeter DR900; Loveland, CO).

Growth

Growth (length (mm) and weight (g) were evaluated weekly for 12 weeks. Five individuals per container ($N=15$/treatment) were sampled in weeks 0–6, and three per container ($N=9$/treatment) in weeks 7–12 due to the decreased quantity of amphipods remaining. Sampled amphipods were preserved in 70% EtOH until analysis. Collected amphipods were blotted dry and weighed using a microbalance (Mettler Toledo LLC, Columbus, OH). A stereomicroscope (Olympus SZX7; Center Valley, PA) with an attached camera (Lumenera Infinity 2) and image analyzing software (Lumenera Infinity Analyze, release 6.5.0; Ottawa, ON) was used to measure length. Individual body length was measured from the anterior of the head to the end of the cephalothorax (segments T1-T8) (Fig. 1) as described by Alegretti et al. [20].

Survival

Survival (%) was assessed at weeks six, 12 and 16. The total number of amphipods were counted in each container, then transferred to new pre-heated containers. Survival (S) was calculated using the following formula: $S = 100 \times \left(\frac{N_f - N_r}{N_i}\right)$ where N_f = final number of individuals, N_r = number of individuals removed to determine growth, and N_i = initial number of individuals.

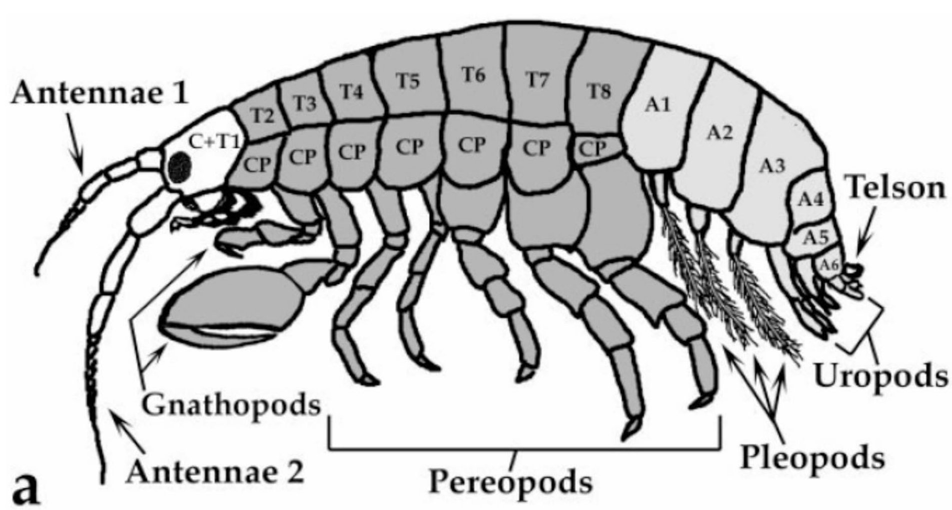

Fig. 1 As depicted in Browne et al. [16], a schematic drawing (**a**) of the adult *Parhyale hawaiensis* body plan. The head (white) consists of the first six segments (termed the cephalon) plus the first segment of the thorax (T1). The pereon (dark grey) is composed of segments T2-T8. The abdominal segments A1–A6 are colored light gray. At the very posterior is the telson, which is a cleft flap of cuticle just posterior and dorsal of the anus

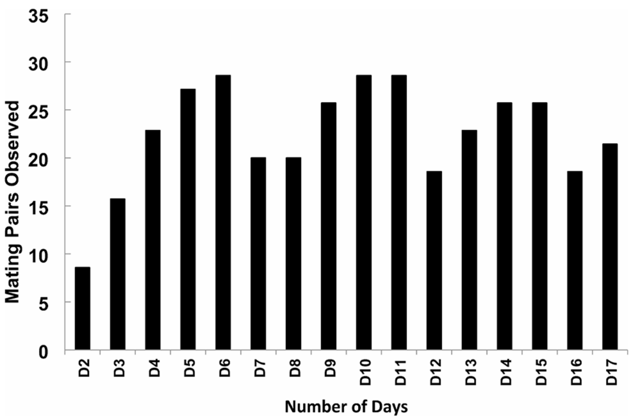

Fig. 2 Number of mating pairs of *P. hawaiensis* observed during daily container ($N = 12$) checks of a potential total ($N = 70–72$) number of pairs

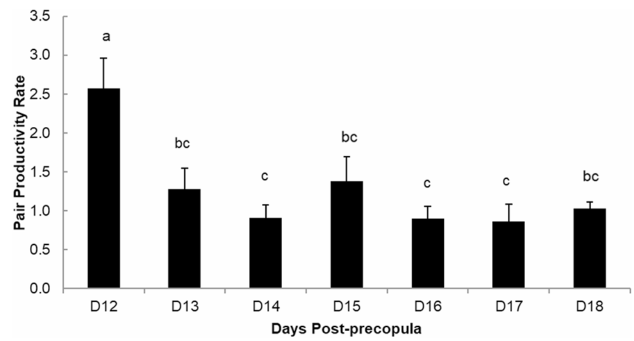

Fig. 3 Mean (±SE) daily mating pair productivity rates of *P. hawaiensis* ($N = 69–71$). Days indicate hatchling productivity following first observed precopula. Significant differences ($P < 0.05$) are denoted by letters (a > b)

Hatchling-to-hatchling generation time

Hatchling-to-hatchling generation time is defined as the amount of time needed for a newly emerged hatchling to reproduce and release offspring. Amphipod containers were visually monitored daily for hatchling production.

Statistics

A one-way analysis of variance (ANOVA) was used to analyze growth and survival data. Percent survival was arcsine transformed before analysis. A Tukey's post-hoc test was applied when significant differences ($P < 0.05$) were found.

Results

Culture establishment

Observed precopula

During the juvenile collection period an average of 22.2 ± 5.6% pairs per day were observed in precopula during daily checks (Fig. 2). One to two mating pairs were observed in four of the 12 containers (8.6% of all potential pairs) on the second day following placement of the females and males into containers. By the fourth day the number had increased to one to three mating pairs observed in 10 containers (22.8% of all potential pairs). From days four to 17, the number of containers in which mating pairs were seen varied between eight to 11. As many as five of six potential mating pairs were observed in precopula in containers during the collection period, although 1–3 was typical. Significant differences ($P = 0.039$) in the number of mating pairs per day was only observed on days two (0.5 ± 0.8) and three (0.92 ± 0.9) compared to days four to 17 (1.33 ± 0.78 to 1.67 ± 0.89). A significant difference ($P = 0.005$) in mating pairs was observed between containers. Three containers had on average less than one pair observed in precopula per day (0.73 ± 0.70 to 0.93 ± 0.26) compared to an average of 1.13 ± 0.56 to 2.13 ± 0.99 in the remaining nine containers.

Survival of brood pairs was high. Mean female survival was 97.2% and mean male survival was 95.8%. Two males, housed in two separate containers died within the first five days, increasing the female to male ratio slightly and thus reducing the number of potential pairs that could occur simultaneously from five to six in those containers. Female mortalities occurred later, resulting in a reduced female to male ratio, but not in the number of potential pairs, as there were twice the number of females compared to males.

Juvenile production

The mean number of juveniles (7.4 ± 4.7) produced per container per day was significantly different ($P = 0.0003$) and ranged from 5.0 ± 4.7 to 14.9 ± 7.9. Highest mean juvenile production occurred 12 days after the precopula was first observed, and lowest mean juvenile production occurred on day 17. The average mating productivity rate per pair was 1.32 ± 0.88 and varied significantly between days ($P < 0.0001$) (Fig. 3). Peak mating productivity was seen 12 days after precopula was first observed, and generally trended lower thereafter. No significant differences were seen between containers.

Temperature study

Water quality

There was no significant difference ($P > 0.05$) between treatments in mean ammonia (TAN) (0.01 ± 0.02 to 0.03 ± 0.06 mg/L), mean nitrite (0.10 ± 0.10 to 0.16 ± 0.16 mg/L), or mean pH (7.9 ± 0.07 to 8.09 ± 0.04) (Table 1). Dissolved oxygen levels differed significantly ($P < 0.0001$) between treatments with the highest levels at 20 °C (7.44 ± 0.15 mg/L) and lowest levels (6.22 ± 0.01 mg/L) at 29 °C. Salinity differed significantly ($P < 0.0001$) between treatments with the lowest salinity (32.8 ± 4.0 ppt) at 20 °C and highest salinity (37.5 ± 3.8 ppt) at 29 °C.

Table 1 Mean (±SD) values of water quality indicators during the 20-week trial period at four temperature treatments. Significant differences ($P > 0.05$) within rows are denoted by superscript letters

	20 °C	23 °C	26 °C	29 °C
TAN (mg/L)	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
NO_2-N (mg/L)	0.11 ± 0.02	0.10 ± 0.01	0.16 ± 0.02	0.13 ± 0.02
pH	7.9 ± 0.07	7.98 ± 0.06	8.02 ± 0.04	8.09 ± 0.04
DO (mg/L)	7.44 ± 0.15^{d}	7.04 ± 0.07^{c}	6.59 ± 0.01^{b}	6.22 ± 0.01^{a}
Salinity (ppt)	32.8 ± 4.03^{b}	36.2 ± 4.11^{a}	36.9 ± 5.03^{a}	37.5 ± 3.82^{a}

Table 2 *Parhyale hawaiensis* hatchling performance in a 12-week trial when maintained at four temperatures. Values (mean ± SD) that are significantly different ($P < 0.05$) within rows are denoted by superscript letters

	20 °C	23 °C	26 °C	29 °C
Final size (mm)	3.7 ± 0.53^{b}	3.5 ± 0.79^{b}	4.4 ± 0.58^{a}	4.6 ± 0.80^{a}
Growth rate (mm/wk)	0.19 ± 0.04^{b}	0.18 ± 0.07^{b}	0.26 ± 0.05^{a}	0.27 ± 0.07^{a}
Final weight (mg)	1.0 ± 0.36	1.7 ± 0.93	2.8 ± 1.39	2.3 ± 0.89
Weight gain (%)	8.2 ± 3.0	14.1 ± 5.7	23.4 ± 11	19.0 ± 7.4

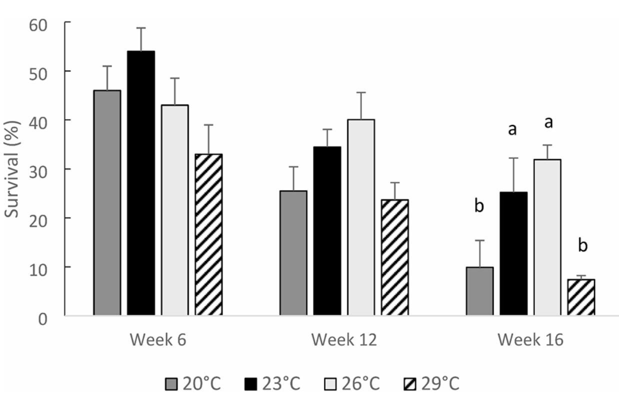

Fig. 4 Mean survival (± SD) of *P. hawaiensis* reared at various temperatures at 6, 12 and 16 weeks post hatch. Significant differences ($P < 0.05$) are denoted by letters (a > b)

Growth

Final body length and weekly growth rate differed significantly ($P = 0.014$) between treatment groups. Amphipods maintained at 26 °C and 29 °C were larger than those in the 20 °C and 23 °C treatments (Table 2). There was a trend towards increased weight at higher temperatures, but differences were not significant ($P = 0.193$).

Survival

Survival at six weeks was highest at 23 °C (54 ± 4.8%) and lowest at 29 °C (33 ± 5.9%), however differences were not significant ($P = 0.122$) (Fig. 4). By 12 weeks survival was highest at 26 °C (40.1 + 9.6%) and lowest at 29 °C (23.7 ± 6.1%), but differences were still not significant ($P = 0.098$) (Fig. 4). At 16 weeks, survival varied significantly ($P = 0.017$) and was higher at 26 °C and 23 °C (32 ± 3% and 25 ± 7%), than at 20 °C and 29 °C (10 ± 5.5% and 7.5 ± 1%) (Fig. 4). At 20 weeks, survival in the treatment groups (20 and 23 °C) that had been pooled (duplicate containers) for continued observation was 65 ± 7.1% and 75 ± 7.1% respectively of that at 16 weeks, which represented 6.6% and 18.9% of the original population.

Hatchling-to-hatchling generation time

Hatchlings were observed in the 26 °C and 29 °C treatments in week 16 with 1 to 10 hatchlings observed per container. The mean number of hatchlings produced at 26 °C ($N = 3$ containers), was 5.3 ± 4.0, and at 29 °C ($N = 2$ containers) was 6.0 ± 5.7. No hatchlings were observed in either the 20–23 °C treatments by week 20, at which time the study was terminated.

Discussion

Studies investigating the aquaculture potential of marine amphipods, *Eogammarus possjeticus* and *Caprella scaura*, have shown encouraging results with respect to population growth under culture conditions [24, 30]. The amphipod *Parhyale hawaiensis* is likewise a promising live food source for aquaculture, yet few studies have been conducted with this species. The present study was undertaken to establish a temperature that would lead to increased growth and survival and decreased generation time in this species. In addition, observations on mating behavior (% in precopula) and productivity rates of mating pairs were collected, to offer insight into the number of males and females required to achieve facility production goals.

Amphipods living under unfavorable environmental conditions may have a higher energetic demand for physiological adaptations, such as osmoregulation rate or molting duration, which consequently hinders reproduction and growth [21]. The effects of temperature on growth and survival of other amphipod species have been quantified, but information is lacking with respect to *P. hawaiensis*. The reported generation time of *P. hawaiensis* varies widely as to the timing (6–20 weeks) and temperature (18–28 °C) at which this occurs [16, 20, 25–27] and are predominately observational.

Rehm et al. [31] stated that *P. hawaiensis* can be maintained at 20–26 °C, with 26 °C being optimal for growth. Although no experimental data was provided to support this, results from the present study agree with those findings. Amphipods cultured for 12 weeks at 26 °C and 29 °C were significantly larger than those reared at lower temperatures. Survival did not differ significantly between treatments until 16 weeks, although there was trend towards lower survival at both the lowest and highest temperatures as the study progressed. By 16 weeks survival at the two temperature extremes was 61–75% lower than that seen at 23 and 26 °C.

Other amphipod studies have shown that temperature impacts growth and survival, but the optimal temperature is species dependent due to species adaptations to the environmental conditions associated with geographic location. Most temperature studies have been conducted with temperate Gammaridae species. Xue et al. [24] found that survival of *Eogammarus possjeticus* decreased when exposed to increasing temperatures ranging from 15 °C to 27 °C, with the highest growth at 21 °C, and similarly low growth at both extremes. In contrast, *E. sinensis* survival was only decreased at the highest temperature [32]. Mortality was also low in *G. locusta* at all temperatures during the first four weeks but increased linearly thereafter, with no survival by week 16 at the highest culture temperature compared to a 22 week survival at lower temperatures [21]. Although final growth followed a similar pattern for both species, at 20 days the specific growth rate of *E. sinensis* increased with increasing temperature [24, 32]. Higher temperatures were also associated with higher growth in *Gammarus locusta*, *Echinogammarus marinus* and the Hyalidae amphipod *Hyale crassicornis* [21, 22, 33]. In addition, higher temperatures were associated with both shortened intermolt length and shorter generation times [21, 22]. Xue et al. [32] suggested that earlier amphipod stages were better adapted to changes in temperature than later life stages. Neuparth et al. [21] concluded that higher temperatures, while resulting in increased growth, also accelerated the life cycle of and thereby reduced the amphipod life span. Although these conclusions may explain the higher growth observed at the two higher temperatures evaluated in the present study, as well as the higher mortality observed at the higher temperature, they do not explain the higher mortality observed at the lower temperature. Water quality did not appear to be an issue. Ammonia and nitrite levels were within normal ranges for aquaculture and were similar between treatments. As expected, salinity was higher and dissolved oxygen levels lower at higher temperatures. Despite weekly water changes, salinity was significantly lower at 20 °C. Although containers were provided with lids, they were loose fitting, providing space for airlines and ease of feeding, which likely allowed for additional evaporation at the higher temperatures. During water exchanges, water levels were simply brought back to the 2 L mark and no additional attempts were made to adjust salinity. Amphipods in general have a wide salinity tolerance [18, 21, 22], and *P. hawaiensis* has been shown to tolerate salinities ranging from 5 to 40 ppt [18], within the 33 to 37 ppt reported in the present study. Despite the lower dissolved oxygen levels at higher temperatures, which would be expected as cold water is known to hold more oxygen than warm water, levels at all temperatures were within acceptable ranges for aquaculture.

Although a trend towards lower survival was seen at the highest temperature by week 6 and at the lowest temperature by week 12, the impact of Hurricane Irma on survival as well as generation time must be acknowledged. Even though aeration was maintained, heaters were disconnected when the FAU-HBOI facility closed, leading to an eventual decrease in temperature in all treatments to ambient room temperature (~ 21 °C). Amphipods maintained at 29 °C would have experienced the most significant temperature impact. However, as no change in temperature would have occurred in the 21 °C treatment, the lower survival seen between weeks 12 and 16 may be explained by other factors. As amphipods were not fed during closure, cannibalism probably contributed to the increased mortality seen. High rates of cannibalism have been reported previously for *P. hawaiensis* with 66–84% of juveniles cannibalized by adults during co-habitation experiments [23]. Although lack of food should have affected all treatments equally, container density varied. All containers were fed a two day ration prior to closure. Replicates with higher densities consumed the available food more quickly, increasing the likelihood of cannibalism. Despite the potential impact of temperature and Hurricane Irma on survival, survival was lower than anticipated at week 6, falling below 55%. To collect samples more easily for growth and observe hatchling production no substrate was provided. This lack of substrate likely increased the potential for cannibalism, as mentioned above, despite the availability of food, leading to decreased survival at all temperatures.

Culture temperature has also been shown to affect generation time. Xue et al. [24] reported that the sexual maturation of *E. possjeticus* female hatchlings was shortened as temperature increased from 15 °C to 21 °C and was lengthened between 24 °C and 27 °C, with optimal offspring production at 21 °C. *Gammarus locusta* reached sexual maturity earlier and had a shorter generation time (six versus eight weeks), but a reduced life span at 20 °C compared to 15 °C, yet the number of offspring produced was not affected [21]. *Hyale barbicornis* matured earlier and at a smaller body size as the temperature increased from 12 °C to 28 °C, however the total number of offspring increased as the temperature decreased to 16 °C [34].

In the present study, a generation time of 16 weeks was established for *P. hawaiensis* at 26 °C and 29 °C. This is close to the estimated time of three months at 24 °C determined for this species by Artal et al. [27] and falls within the three to five month period calculated for natural populations [20]. It is, however, higher than the 6 to 8 weeks reported for *P. hawaiensis* reared at temperatures ranging from 18 °C to 28 °C [16, 23, 25, 26]. While *P. hawaiensis* can produce embryos every few weeks, each female produces only a small number at a given time.

Reported female fecundity of *P. hawaiensis* ranges from 1 to 27 embryos with an average of 6 to 13 [16, 23, 27, 35]. Reasons for these reported discrepancies may include variations in density, sex ratio, nutrition, and water quality, and in the case of the present study all but water quality may apply.

By the time hatchlings were observed (week 16) replicate containers contained from three to 16 amphipods each, far less than the 18 amphipods (2:1 female to male ratio) used to produce juveniles for this study. Yet Vargas-Abundez et al. [23], were able to obtain hatchlings with as little as ten adult amphipods per container at a 4:1 male to female ratio. Unfortunately, the sex ratio in the replicate containers in this study was not evaluated. One replicate (29 °C) produced hatchlings at 16 weeks with as few as five amphipods, indicating that despite a small number of amphipods, a sufficient sex ratio existed for hatchling production to occur. However, it should be acknowledged that this may not have been the case for other containers that also had low numbers of amphipods, which would have accounted for lack of hatchling production.

Hatchlings may have been produced between weeks 12 and 13, when the facility was closed, and either did not survive due to lack of food or were cannibalized. Egg quality and therefore embryo survival may have been negatively affected due to a combination of thermal stress and lack of food, reducing female fecundity and lengthening observed generation times after that time. Artal et al. [27] reported an 89% embryo hatching success rate for *P. hawaiensis*, a somewhat higher, but comparable rate to that (70–80%) reported for other amphipods [36]. However, it is assumed that amphipods were reared under optimal conditions.

In addition to the lack of food, which would have played a role in both survival and generation to generation time after week 12, nutrition could have played a role in determining generation time before that time. In previous amphipod studies diets offered consisted of various macroalgae species [21, 24, 32] or commercial fish and shrimp diets [23, 33]. All diets appeared to be adequate for culture and rearing studies, although none, except for Vargas-Abúndez et al. [23], compared diets. In that study, no effect was seen with respect to fecundity, although a slight difference was seen in growth, with juveniles fed the plant based diet having increased total length. Biofloc, used as the sole food source in this study, proved to be adequate for a previously *P. hawaiensis* study (unpublished data). Compared to commercially available diets, macroalgae and biofloc contain high protein levels, but a much lower lipid content ([37], in review). *P. hawaiensis* fed a commercially available shrimp feed had 4.5 times the density at four weeks compared to those fed an *Ulva lactuca* diet indicating that the macroalgae diet may have provided inadequate nutrition for reproduction [38].

A low productivity rate of *P. hawaiensis* and longer generation times compared to other amphipods, such as *Caprella scaura* has led to the conclusion that other amphipods species may have better aquaculture potential [23]. Although a long generation time, especially at lower temperatures, was also found in the present study, dietary studies conducted with *P. hawaiensis* ([37, 38] in review) show good production. The previously reported productivity rate for *P. hawaiensis* of less than one (0.36) juvenile per mating pair [23], is much lower than that found in this study (1.32), and closer to the calculated productivity rate of 1.03 for *C. scaura* [23]. The main difference between this study and that of Vargas-Abúndez et al. [23] is that the number of juveniles was assessed every day in the present study versus at 15 and 30 days in that study. As high mortality of juveniles in that study was noted and attributed to cannibalism, it is highly likely that more frequent assessment in that study would have resulted in a much higher productivity rate. This higher productivity rate, despite a potential longer generation time, argues in favor of the aquaculture potential of *P. hawaiensis*.

Conclusion

The results of this study show that temperature significantly affects growth, survival, and generation time of *P. hawaiensis*, and suggests that *P. hawaiensis* production is enhanced at 26 °C. This information, along with data obtained concerning mating productivity rates may be used to further optimize conditions to advance the development of large-scale culture of this species. Future studies are needed to determine in combination the optimum temperature and feed type to enhance the reproduction of this species.

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Author contributions

S.L. designed the study, analyzed the data, and prepared the figures and tables. E.A. conducted the study, collected the data and prepared the manuscript. S.L. reviewed and edited the manuscript. Both authors participated in manuscript preparation and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval
All applicable international, nation and institutional guidelines for the care and use of animals were followed.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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