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Population dynamics of *Parhyale hawaiensis* (Crustacea: Amphipod) in laboratory culture and life history differences between laboratory and wild populations

Dinâmica populacional de *Parhyale hawaiensis* de uma cultura de laboratório e comparação de história de vida com uma população natural

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Área de concentração: Tecnologia para o Ambiente.

Orientadora: Prof.ª Dr.ª Maurea Nicoletti Flynn

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A Banca Examinadora composta pelos membros abaixo aprovou esta Dissertação.

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* A Ata da defesa, com as respectivas assinaturas dos membros, encontra-se no processo de vida acadêmica do aluno.

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RESUMO

Ramos da ecologia, tal como a teoria da história de vida, podem contribuir para a expansão do espectro de atividades da Ecotoxicologia clássica englobando o poder preditivo de alguns dos princípios da ecologia populacional. Este trabalho se propõe a analisar as estratégias demográficas adotadas pelo crustáceo marinho *Parhyale hawaiensis* mantido em laboratório, descrevendo seu ciclo de vida, fitness reprodutivo e dinâmica populacional através da construção de tabela de vida tempo-especifica; e comparar os dados obtidos com aqueles para populações naturais da mesma espécie. Considerando populações criadas em laboratório ou na natureza, a espécie se reproduz praticamente o ano inteiro. As fêmeas têm reprodução continua (iteróparas), e exibem ciclo de vida multivoltínico. Comparadas a populações naturais oriundas do mesmo local, populações de *P. hawaiensis* mantidas em laboratório apresentam reprodução tardia, fecundidade mais alta associada ao corpo maior, mortalidade mais baixa, e maior tempo de geração; tem vida mais longa, ao menos 20 meses (e 25 meses estimados) e parece apresentar senescência no período final da vida, comportamento claramente K-estrategista, contrastando com populações naturais, claramente r-estrategistas. O período de vida mais longo em laboratório parece associado a um número maior de mudas, mas não diretamente com o tamanho do indivíduo após este atingir o tamanho de 7 mm. Deste tamanho em diante as mudas parecem ser somente relacionadas a reprodução. O cultivo de *P. hawaiensis* em laboratório fez com que aumentassem, em comparação as taxas exibidas por populações naturais, os valores do potencial reprodutivo R_0 de 2,85 para 63,2 número de filhotes por fêmea, o tempo de geração de 4,54 a 12,1 meses e a taxa intrínseca de crescimento de 0,27 a 0,34 per capita por mês. Conclui-se que os potencias endpoints demográficos como taxa instantânea de crescimento (ri), potencial reprodutivo (R_0) e tempo de geração (T) não parecem ser efetivos para populações de *Parhyale hawaiensis* cultivadas em laboratório. Parâmetros como taxa de sobrevivência (l_x), fecundidade (m_x), idade de maturidade sexual e capacidade suporte (K) poderiam ser mais eficientes endpoints se associados a taxa intrínseca de crescimento (*r*).

Palavras-chaves: parâmetros demográficos, ecotoxicologia, Amphipoda

ABSTRACT

Branches of ecology such as life-history theory, can fully contribute in the expansion of the spectrum of activity of classical ecotoxicology by encompassing the predictive power of population dynamics principles. This work sets out to provide detailed baseline data on the population dynamics of *Parhyale hawaiensis* kept in laboratory, describing the life cycle, fitness parameters and population dynamics of a culture through the construction of time-specific life table for obtaining population rates related to reproductive success; and comparing data obtained from a wild population of the same species. *P. hawaiensis,* considering wild or lab populations, reproduces almost year-round. Females produce broods consecutively (Iteroparity), exhibiting a multivoltine life cycle. Lab *P. hawaiensis* population compared to the wild population extracted from same location presented a delayed reproduction, higher fecundity associated with a slightly larger body size, experiences lower mortality and has a longer generation time; long-lived for 20 months with estimated complete decay at 25 months because it appears to experience senescence, representing a classical K selection. In contrast to the classical *r* selection strategy evidenced for wild populations. Longer life span in, apparent in laboratory, is coupled with a larger number of moults. However, this much larger life span is not directly related to individual's size after individuals attained approximately 7 mm. From this size on it seems that moulting in female is related exclusively to reproduction. The cultivation in laboratory of *P. hawaiensis* has raised the net reproduction rate R_0 from 2,85 to 63,2, young per female, the time generation from 4,54 to 12,1 months and the maximum intrinsic rate of increase *r* from 0,27 to 0,34 per capita per month. In conclusion, demographic endpoints such as instantaneous growth rate (ri), net reproductive rate (R_0) and generation time (T) don't appear to be effective for lab populations of *Parhyale hawaiensis*. Perhaps parameters such as survival rate (l_x), fecundity (m_x), sexual maturity achievement size and carrying capacity (K) could be more efficient endpoints, when coupled to the intrinsic growth rate (*r*).

Key Words: demographic parameters, ecotoxicology, Amphipoda

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SUMMARY

INTRODUCTION

Aquatic fauna has been used as test organisms in ecotoxicological assessments supported by the assumption that any ecological phenomena is constrained by the organisms involved and their ecological environment. The classical concern of ecotoxicology with experimental tests, dose-effect analysis and estimation of effect concentrations has provided a solid basis for environmental regulation on maximum acceptable chemical concentrations. Nevertheless, this approach does not stimulate the incorporation of ecological theory, indispensable to fulfill the current need to assess toxicants effects on natural populations and ecological systems (FLYNN & PEREIRA, 2013).

Branches of ecology such as life-history theory, can fully contribute to expand the spectrum activity of classical ecotoxicology by encompassing the predictive power of population dynamics principles such as the exponential growth (Malthus equation), the self-limiting population (Verhust-Pearl logistic growth model), and the consumer-resource oscillation (Lotka and Volterra classical predator-prey system) (TURCHIN, 2001). Therefore, a tool to evaluate the frequency and magnitude of impacts can be created by integrating population-level endpoints as a more realistic and ecologically relevant measure of toxic response. For that, general concepts of standard patterns, replicable rules of population ecology and predictive modeling must be applied (WALTHALL & STARK, 1997; VAN STRAALEN, 2003).

Life history theory attempts to understand the variation in such life-course variables as gestation length, age at sexual maturity, litter size, inter-birth interval, body size, and adult and juvenile mortality rates. The life strategy concept postulates that natural selection molds the occurrence and duration of key events throughout the life cycle in order to optimize the survival of the offspring. Changes in life history traits are interpreted in terms of Darwinian fitness selection, which is merely the total number of offspring produced by an animal over its lifetime, in a controlled environment. Age and size of sexual maturity, each reproductive event, reproductive success, senescence and death all depend on ecological conditions

to which the organisms are submitted over time, determining its evolutionary path (TOWNSEND, HARPER & BEGON, 2010).

Within the theories on life strategies, a highly supported theory is the r-K strategy. K strategy is associated to a high survival rate both for adults as to young individuals of a population. In this strategy, females present low fecundity, long generation time and are iteroparous (breeds more than one offspring during its life), maintaining population densities relatively stable in this way. In addition, *r*-strategy is characterized by a low survival rate amongst adults, high fecundity, iteroparous females and short generation time resulting in a more inconstant (variable) population density (MACARTHUR & WILSON, 1967; PIANKA, 1970; STEARNS, 1976).

Population fitness can be defined in a qualitative way as the ability of a population to maintain itself and/or grow in a particular environment (LEWONTIN, 1957), it is clear that variation in fitness may involve variation in both survivorship and fecundity. Growth and reproductive success are amongst the most important Darwinian fitness traits for species' survival. As such, significant impairment of growth and reproduction will lead to a decline in natural populations, thus threatening species survival and genetic diversity (WU & OR, 2005).

The study of life history traits is essential to understand the population's biology and the ecological role of a particular species (RUMBOLD, 2015), predicting how organisms should assign resources to growth, survival and reproduction in such a way as to maximize their reproductive success or population's fitness (STEARNS, 2000). The major components of fitness are affected by many morphological and physiological traits, correlated with each other on the genetic level (DOYLE & HUNTE, 1981). So when environmental conditions change, altering the fitness or subsistence, organisms may change their patterns of resource allocation shifting their reproductive strategy or growth rates, in order to maintain reproductive success (DUFFY & THIEL, 2007).

An important step to promote ecotoxicological studies is to find an appropriate model organism that can help both acute and chronic toxicities with reproductive, standardized and comparative results on individual and population levels. Determining the life cycle parameters and evaluating fitness impairment of

a particular species creates possible integrated endpoints, through the establishment of vital intrinsic rates or parameters, to be used comparatively on ecotoxicological studies. Toxicity is then assessed by measuring the deficits that occur in population survival, growth, and reproduction with exposure to a chemical when compared to its normal survival, growth, and reproductive capacities without that chemical stressor. It is expected that populations of the same species exposed to dissimilar conditions presents altered life history traits (STEARNS, 2000). These 'normal' levels of survival, growth, and reproduction under a given set of conditions are referred to as life history characteristics (MAJOR, 2012).

With the continuous development of ecotoxicological tests towards higher ecological relevance, life-table analysis has been used (LEVIN et al., 1996; CONRADI & DEPLEDGE, 1998 and 1999; FORBES & CALOW, 1999). The construction of life tables to obtain derived vital parameters such as: fecundity, viability, generation time, sex ratio, net reproductive rate, survival rate and intrinsic rate of population growth, create the possibility to calculate and evaluate the frequency and magnitude of a variety of environmental impacts (FLYNN & PEREIRA, 2009 and 2011; FLYNN et al., 2008 and 2009). These parameters may be used as beacons signaling alterations related to the environment the organisms are inserted, opening the possibility to study with higher precision the effects of natural or synthetic substances on a population. This is an important step since several studies indicate that barely perceptible sub-lethal effects affect a population in lower concentrations in comparison to dose response curve tests, used traditionally to obtain individual level *endpoints* (NORBERG-KING et al., 2006; STARK & BANKS, 2003; DEVAUX et al., 2011).

Life tables can be time-specific or age-specific. It is more relevant to use the construction of a time-specific life table, obtained by following the development and survival of one cohort (individuals born together in the same moment or short time frame), commonly accomplished in laboratory (WALTHALL & STARK 1997; FORBES & CALOW 1999). While age specific life tables are constructed with *in situ* data (FLYNN et al., 2008 e 2009; ALEGRETTI, 2015).

Amphipods, as one of the most abundant and diverse groups of marine benthic animals found in coastal waters worldwide, serve as an important trophic

link from primary producers to higher order consumers and play an important role in nutrient recycling of marine coastal systems. Conceivably, any adverse impact on amphipods may lead to major ecological consequences for ecological functions, including alterations in energy flow and disruption of nutrient recycling processes in coastal ecosystems (WU & OR, 2005).

Life-history patterns of gammarid amphipods are influenced by latitude, depth and salinity (SAINTE-MARIE, 1991), with low latitude species tending to breed throughout the year and having short life spans (MORINO, 1978). According to Cunha et al. (2000) low-latitude, warm water amphipods show iteroparous, multivoltine life history patterns. Tropical species are characterized by small size, low fecundity, short brood intervals and multivoltine life cycle (STEELE & STEELE, 1991) while, low-latitude species are characterized by semi-annual or annual life histories, small body size and high reproductive potentials (SAINTE-MARIE, 1991).

Parhyale hawaiensis has been used sporadically for toxicological and ecological studies. The species present proper characteristics for a model species i.e. is abundant, largely distributed, ecologically relevant and manageable in laboratory experiments (RAISSUDDIN et al., 2007). Vulnerable stages, for example, such as hatching time and juvenile growth are among the most adequate test parameters (LACAZE et al., 2011). Now, a concerted effort has been made to establish *P. hawaiensis* as a new crustacean model organism for studying the relationship between development and evolution. Several aspects of its life history make this particular species amenable to many types of classical and modern laboratory analyses and techniques (BROWNE et al., 2005; REHM et al., 2009).

Individuals of *P. hawaiensis* are detritivores and have a circuntropical, worldwide, intertidal, and shallow water marine distribution (EXTRAVOUR, 2005), possibly as a complex species (MYERS, 1985). They have been reported to aggregate in large populations (>3,000/m^2) in environments subjected to rapid changes in salinity (POOVACHIRANON, 1986). In laboratory conditions, females breed their young in a ventral pouch and produce embryos every 2 weeks once they reach sexual maturity. The length of embryogenesis is relatively short, lasting roughly 10 days. Adults are relatively small (<2cm) and easy to maintain, even in

dense laboratory cultures. When handled, individual broods of synchronous eggs can be easily removed from females, and then, kept in small volumes of seawater until hatching. Gammarids go into a pre-copula position before egg laying, the female moults and the eggs drop from the oviducts to be stored in the brood pouch. The male then externally fertilizes the eggs from the female's brood chamber (GULER, 2012). Adults breed at every moult change that occurs in approximately 2-3 weeks (BROWNE, 2005; REHM et. al., 2009).

Demographic *endpoints*, covering parameters such as survival rate (l_x), fecundity (m_x), sexual maturity achievement size, carrying capacity (K), extinction probability (ór^2> 2 m_{ean}) were established for a wild *P. hawaiensis* population by Alegretti (2015) as *endpoints* for chronic toxicity tests. Thus recommending the application of intrinsic growth rate (r), net reproductive rate (R_0) and generation time (T) as more efficient *endpoints*. Results showed that this wild population, located in the intertidal region of Itanhaem's rocky shore, in southeastern Brazil, produces a small numbers of eggs per brood correlated to a decrease in the size of maturing females, which in turn enables the production of more than one offspring per year, increasing the intrinsic growth rate of the population. This is an ***r*** strategist, with continuous reproduction, multivoltine cycle and recruitment throughout the year, reported also for many other tropical amphipod species (CUNHA et al., 2000; CHANDANI & ALAN, 2004). The population kept in LEAL lab culture was derived from individuals of this same wild population.

Such life history parameters compose the basic information of paramount importance if a population level approach is to be effectively used in ecotoxicology (Paull et al., 2008). Although the present great scientific interest on *P. hawaiensis*, only few studies have focused on life history parameters of the species, and the available data, as we have seen, was assessed from wild samples. Wild population endpoints are very hard to use as toxicity presumption for a lab population model as very little is understood about the potential life history differences between wild and lab kept populations. It is important to, preceding toxicity testing, establish the differences between populations in endpoints measured otherwise, results for chronic toxicity tests may not indicate so much the effect of a given chemical as much as the inherent characteristics of the species environment (BEERMAN & PURZ, 2013).

An understanding of the connection between demographic traits and toxic response is still deficient for test organisms associated to laboratory and wild populations. Differences already pointed out under laboratory conditions are altered adult intermoult period that usually becomes longer for crustaceans over time, related to age and/or size of the individual (CONAN, 1985) and longer lifespan for adult females (BEERMAN & SPURZ, 2013). It is important to stress that the process of moulting is essential for brood production in amphipods, as oviposition only can take place immediately after ecdysis. Shorter intermoult periods thus result in a quicker succession of broods and should promote population growth. Reproduction, growth and moulting are interlinked and subsequent to hatching (SHEADER & CHIA, 1970). Wild studies have demonstrated that many amphipod species are sensitive to environmental parameters interfering in the percentage of egg bearing females, population density, sex ratio, egg volume and fecundity (GULER, 2012).

There is no investigation about life history parameters of *P. hawaiensis* under controlled laboratory conditions. The variation already documented between amphipod populations in the wild and in the laboratory has the potential to be especially problematic in the context of toxicity testing (MAJOR, 2012). Alegretti (2015) emphasized the need to refine, through lab kept cultures, the parameters established for the wild population. Therefore, it is important to quantify the wild versus lab demographic parameters' differences before attempting to quantify toxicity on population-level.

This study sets out to provide detailed baseline data on the population dynamics of *Parhyale hawaiensis* cultured in laboratory, information that is scarcely available for this model species. Describing the life cycle, fitness parameters and population dynamics of a culture, through the construction of time-specific life table acquires population rates related to reproductive success. The experimental approach used in this study may serve as a basis for implementation of life-table analysis in long-term tests with *P. hawaiensis*, aiming to truly assess population-level responses to toxicants. Attending to the suggestion by Alegretti (2015) of refinement of specific endpoints, obtained through the construction of age-specific life table for a wild *P. hawaiensis* population, such as the intrinsic rate of increase (r), net reproductive rate (R_0), and generation time (T), those

demographic parameters will be quantified for a lab kept population. Modeling of these rates can be used as comparative endpoints of potential long-term effects of toxic agents for ecotoxicological studies considering wild and/or lab populations.

MATERIAL AND METHODS

Amphipod collection and maintenance for laboratory culture

The initial specimens were collected at Poço do Anchieta Beach, at the city of Itanhaem, São Paulo (Figure 1), between January 2010 and December 2011. An official identification of the collected material documented by the amphipod specialists Fosca Pedini Pereira Leite (PhD) and Silvana Gomes Leite Siqueira (PhD) from the Animal Biology Department from the Biology Institute of Unicamp confirmed that the species cultures as being *P. hawaiensis*.

Figure 1. Sampling collection area: A - Overview map of the region (source: Google Earth), B – Approximated overview of sampling area (source: Google Earth) e C – Picture taken of sampling site.

In the laboratory, individuals of *P. hawaiensis* were sorted out and kept in tanks with recirculation system for one month, to acclimatize to experimental

conditions. Organisms were kept in lab conditions following Cassandra Extravour (2004) *Parhyale hawaiensis* culture notes, with all necessary adaptations made.

The laboratory of ecotoxicology and environmental microbiology, Professor Dr. Abílio Lopes (LEAL), at UNICAMP Technology College (Faculdade de Tecnologia da UNICAMP), has been working with cultures of *Parhyale hawaiensis* population for more than 3 years. The cultures are maintained in glass containers (4L capacity) filled with 2,5L of reconstituted salt water at 30-34 ppt. salinity, 1 cm of inactivated rock as substrate distributed through the whole bottom of the container, constant aeration and photoperiod of 16 hours of light to 8 hours of dark.

To prevent fungus proliferation, all substrate are sterilized before use, aeration air pumps are frequently checked for normal functioning, and rubber tubing and air stones frequently cleaned. Incubators allow temperature to be conditioned between 23-25°C.

Photoperiod light intensity using fluorescent 40W lamps may vary between 500 to 1000lux. Maintenance is made whenever necessary, varying from 1 to 2 weeks depending on population density. Very dense populations require more care as they foul the water faster. Food is provided two times a week, ⅓ of fish food pellet is given to each culture tank. Up to this moment 10 culture tanks are kept in the laboratory (Figure 2).

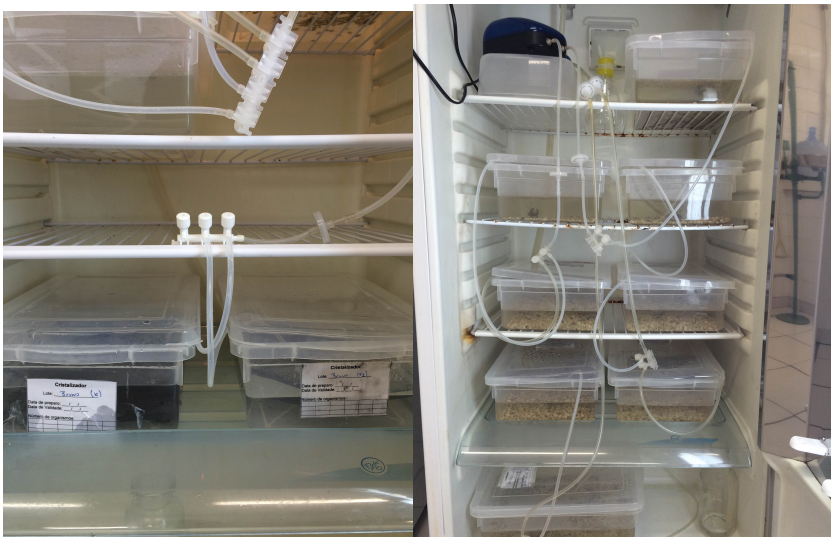

Figure 2. Culture tanks with *P. hawaiensis* at LEAL lab in Limeira, SP.

Pairs (males and females) were then isolated and transferred to separate beakers, each containing 200 ml of marine water not aerated with nylon nets as artificial substrate. Temperature was maintained at 24 ± 1 ºC, salinity 32 ± 2 and pH 7.4-8.6. A few strands of dried algal matter were added to each bowl as food for the animals. These beakers were labeled for later identification (Figure 3). The set of individuals released from a brood was nurtured together in separated bowls for further experiments, as shown below.

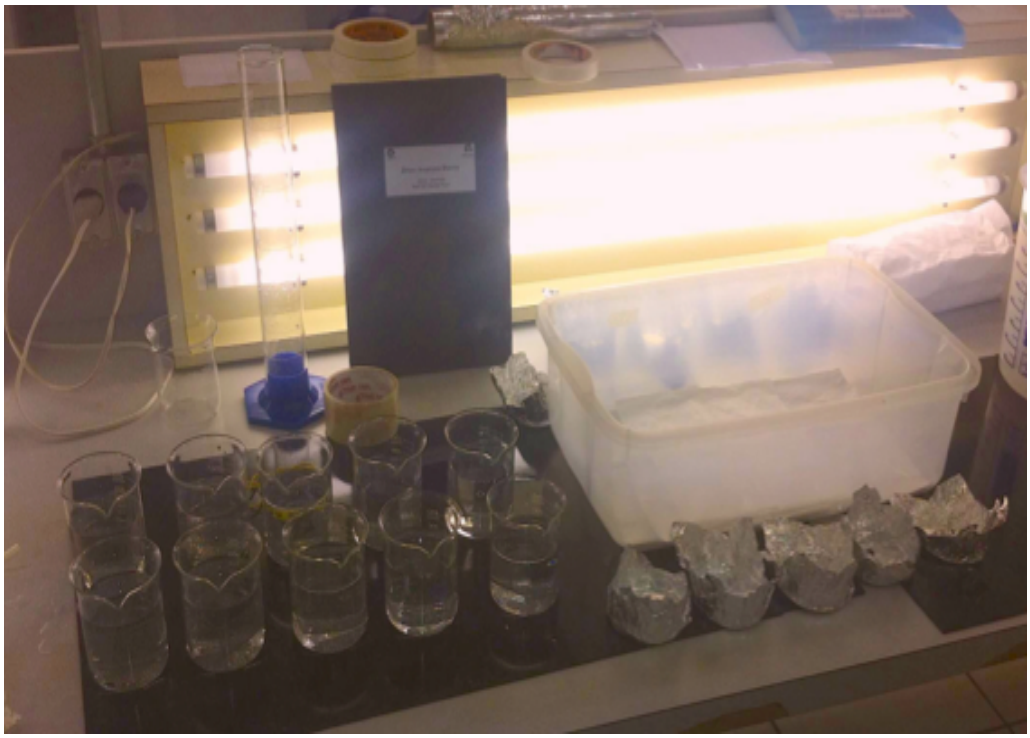

Figure 3. Experiment initiation, couples in copulatory amplexus placed into separate beakers.

Life Cycle Experiment

To gather up data on the species life cycle the experimental manipulation has been conducted with a daily follow up. In early June 2014, to begin the experiment, embryos from sexually mature pairs of *P. hawaiensis* already in copula amplexus (Figure 4), were randomly select from the 10 already existing culture tanks. Each chosen couple was removed and placed in separate beakers containing approximately 200mL of prepared artificially reconstituted salt water with the minimum substrate needed. Since water cannot be aerated during these

procedures, it is aerated before the initiation of these tests and prior to every water change. Water and culture maintenance will occur every 2 to 3 days. All other culture procedures follow the methodology described above.

Figure 4. Male (left) and female (right) of *P. hawaiensis* in copulatory amplexus.

After male fertilization occurred and the female was released from the copula amplexus, the male was removed from the beaker leaving only the female. Due to random selection applied when sorting couples already in amplexus, not all females released their eggs at the same time. Therefore, in order to obtain a more precise and cohesive data, the 10 females that release their offspring in the nearest time interval (at most 3 days) were the ones used as parental generation. Their brood was called First Generation (G1). This makes the construction of time-specific life table more efficient because all individuals belong to the same class.

After the juveniles (G1) reached a certain maturation stage, they were put together and kept in culture as a cohort, meeting all methodological procedures of the laboratory culture described above. In order to cover thoroughly all stages of the amphipod life cycle the experiment followed the cohort until the death of the last individual (Figure 5).

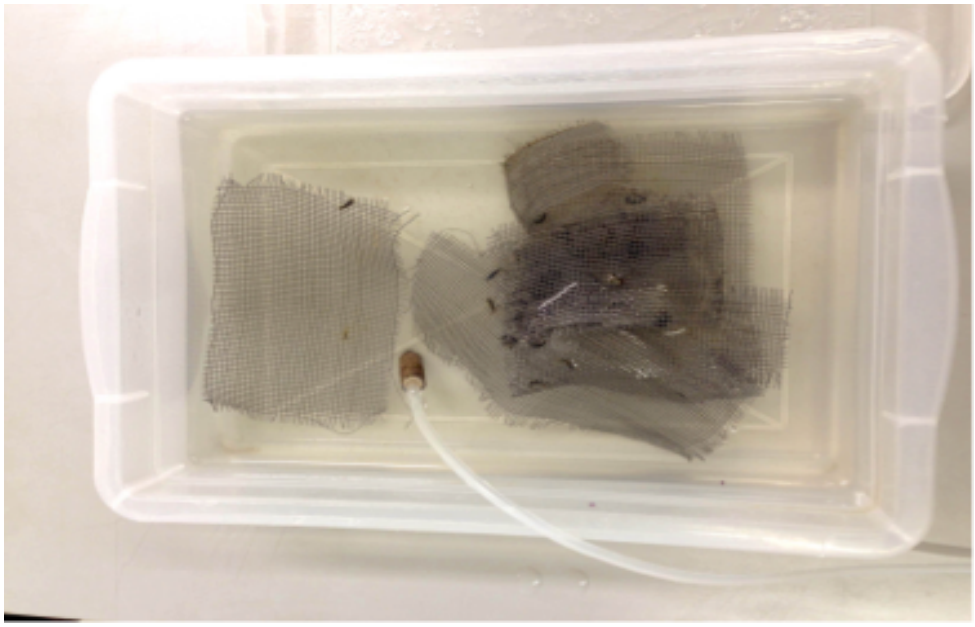

Figure 5. Culture tank with the cohort for the life cycle experiment.

Growth rates were assessed as the average length increment in body size. Live animals were removed from the culture bowl soon after their release from the brood pouch and measured for total length at regular intervals. An ocular micrometer was used for the body length measurements. Body size length was measured as the distance from the rostrum (base of insertion of the first pair of antennas) to the tip of the telson, in millimeters. Cephalothoraxes length was measured as the distance from rostrum to the final segment of the cephalothorax (7^{th} abdomen segment), in millimeters. Growth rate measurements were carried out with a stereoscopic magnifier with a camera attached, with the aid of the software image tool. Photos were taken of each individual in a Petri dish containing minimum water necessary. The image data was uploaded to a computer (Figure 6) and measurement of each individual was done with Imagetool 3.0.

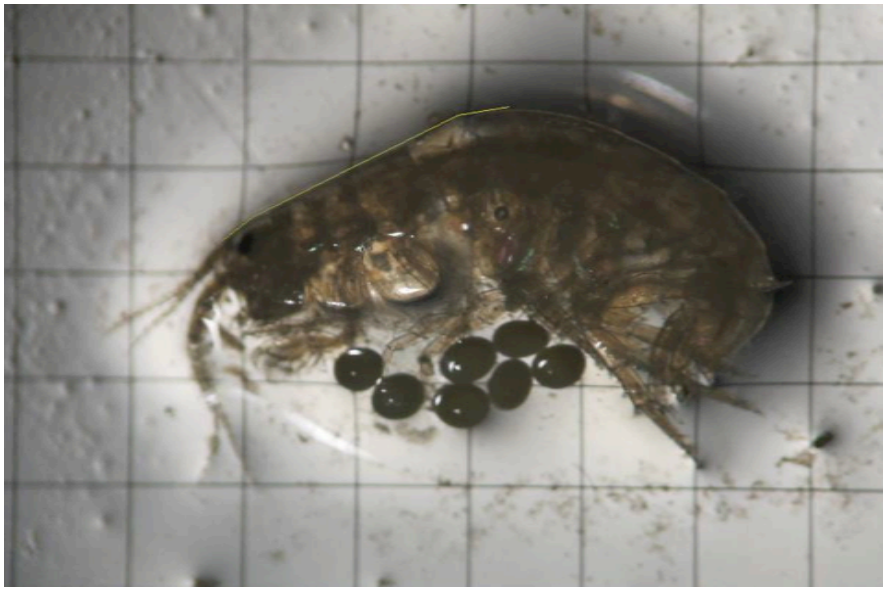

Figure 6. Measurement of each individual with Imagetool 3.0 (Source: Alegretti, 2015).

Survival rate was determined based on offspring mortality, for each moult stage and for each female on G1. The construction of life table is based only on females. The initial size of each generation (N0) was standardized for an initial number of 1000 female individuals (FLYNN, VALÉRIO-BERARDO & PEREIRA, 2008). In this way values of the first column (*Nx*) that represents the number of female survivors for each corresponding group, were obtained. The second column (*Sx*) represents the proportion of survivors (or survival rate) between the respective class and the previous one and it is calculated by the equation *Sx* = *Nx*/*Nx*-1. The third column (*lx*) refers to the proportion of survivors in relation to the initial (*N0*) class and is calculated by the equation: *lx* = *Nx*/*N0*. The fourth column (*Dx*) represents the number of individuals that have died between each class in relation to the previous class, its equation is: *Dx*=*Nx* + (Nx+1). The fifth column represents the probability of death (or death rate) between a class and the one previous to it (*qx* = *Dx*/*Nx*) (GOTELLI, 2007).

After the construction of the life table, a fecundity (*mx)* table was achieved. Information on fecundity (mean number of eggs per female in each class) was obtained distributing the total number of neonates at each class by the number of females present.

The net reproductive rate (R_0) was calculated by the total sum of the product of survival rate (*lx*) and birth rate (*bx*) of each size class (*x*) expressed by:

$$R_0 = \Sigma l_x b_x.$$

The generation time (T) was calculated as:

$$T = \Sigma l_x b_x x / \Sigma l_x b_x.$$

The intrinsic rate of population growth (*r*) was calculated as:

$$r = \ln (R_0)/T.$$

Sexual maturity and sex ratio

In 06/06/2014, newly born juveniles from a single brood were separated in a bowl and reared together. The day at which the female was found to be ovigerous was recorded. Number of males and females were counted at the first data when sex identification was possible (7/08/2014) to find out the sex ratio. Sexual dimorphism of *P. hawaiensis* as described by Browne 2005, body plan of mature males were identified based on the presence of an enlarged second gnathopods and females based on the presence of brood pouch/lamellae.

Following population growth and establishing the instantaneous rate of population growth

Following the growth of a population allows us to estimate the environmental Carrying Capacity (K) according to the laboratory standards and procedures and to create the Logistic Curve for *P. hawaiensis*. For this experiment, 10 couples, paired in copulatory amplexus were randomly selected from the laboratory pre-existing cultures. They were placed together into the same 2,5L container, and the same laboratory culture procedures were performed throughout 6 months. During this time, along with the parental generation (P1), all derived broods and offspring

were kept, therefore maintaining (F1, F2, F3…etc.) generations together, therefore allowing, generation overlapping. Observations for counting number of individuals and establishing sex ratio were done weekly.

Through this experiment, we were able to calculate the Instantaneous Rate of Population Growth (*ri*). A monthly interval was established in order to better express (*ri*) comparative values similar to Alegretti 2015, who made monthly samples. As Walthall & Stark (1997) equation suggests, *ri* is calculated upon the number of individuals per time interval (*Nt*) by the number of individuals at the first time interval (*N0*), applying a natural logarithm to it and dividing this result by the time considered (*t*).

$$ri = \frac{\ln(\frac{Nt}{N_0})}{t}$$

By following the population growth in a restrained given space and energy source, it is possible to observe the maximum population density in one specific container, i.e. the Carrying Capacity (K) and Intrinsic Rate of Population Growth (*r*).

The logistic curve is then obtained through the equation:

$$N(t) = \frac{K}{\left[1 + \frac{K - No}{N0}\right] e^{-rt}}$$

Where as:

N = Number of individuals

t = Time

K = Carrying capacity

r = Intrinsic rate of population growth

RESULTS

Life cycle and growth experiment

From the ten parental couples selected as potential cohort precursors, 8 females were fertilized by their respective male, generating a total of 116 juveniles. The maximum number and minimum number of juveniles born were 23 and 8, respectively, with a mean number of 14,5±4,9 juveniles per female (Table 1).

Table 1. Initial number of juveniles hatched by each parental female and mean juvenile per female.

Female	1	2	3	4	5	6	7	8	Total	Mean	Standard Deviation
Number of Juveniles	14	23	8	17	15	11	10	18	116	14,5	4,9

Growth rate

Almost all neonates (94 in 116) were measured in date 06/06/2014, considering both, cephalothorax and full body size, right after they were released from the female marsupials. Newly born juveniles presented a cephalothorax minimum size of 1,272mm and maximum size of 1,981mm and a mean size of 1,577mm.

The growth perspective divided in months for the first year demonstrates a tendency of a very rapid growth at the begging of the development. The first time interval presented a growth rate of 0.05 mm day-1, and this tendency diminished slowly until the fourth month 0,027 mm day-1 (Table 2). From the fifth month on, growth rate never surpassed 0,004 mm/day, meaning ten times lower than the rates presented on the first two time intervals.

Table 2. Cephalothorax minimum, maximum and mean size (mm) for each month interval. Monthly and daily size growth rate.

Date	Month	Min. Size	Max. Size	Mean Size	Size Growth /month	Size Growth/ day
06/06/14	1	1,272	1,57	1,57	1,554	0,0518
02/07/14	2	2,252	4,199	3,124	1,394	0,0465
07/08/14	3	3,8	5,269	4,518	1,002	0,0334
04/09/14	4	5,078	6,128	5,52	0,822	0,0274
06/11/15	5	5,64	6,942	6,342	0,099	0,0033
04/12/15	6	5,85	6,991	6,441	0,109	0,0036
08/01/15	7	6,026	7,04	6,55	0,197	0,0066
06/02/15	8	6,268	7,61	6,747	0,136	0,0045
05/03/15	9	6,442	8,09	6,883	0,088	0,0029
01/04/15	10	6,626	7,89	6,971	0,119	0,0040
01/05/15	11	6,752	8,12	7,09	0,061	0,0020
03/06/15	12	6,79	8,197	7,151	-	-

The lab population presented a logistic growth curve (Figure 7). Growth rate was higher in early developmental stages slowing down at later stages.

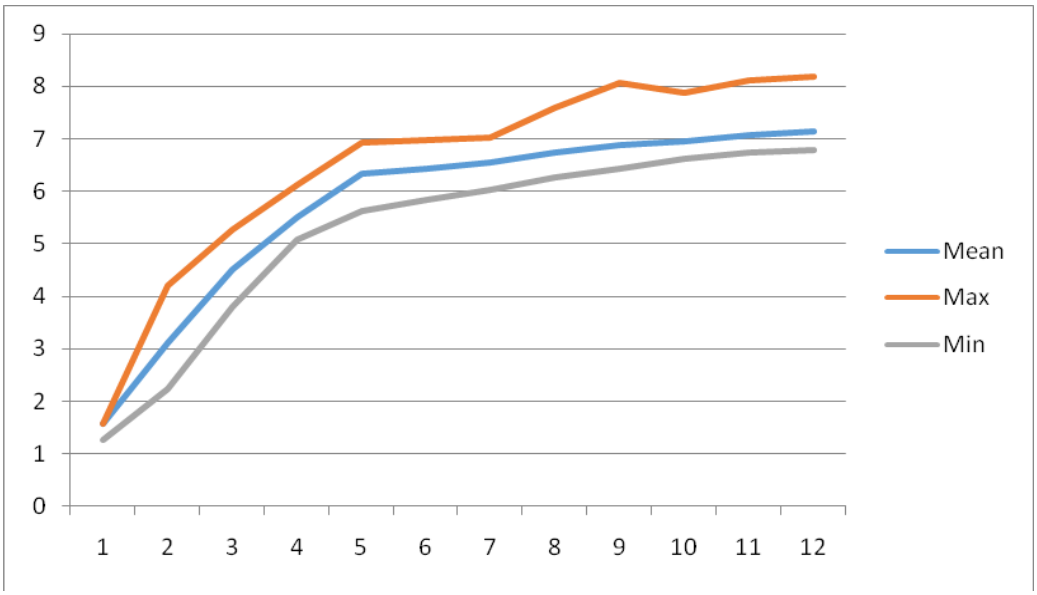

Figure 7. Growth curves elaborated with the minimum, maximum and mean sizes of individuals in the first year of life. Time (x axis) is represented by months and growth (y axis) in millimeters.

Attainment of sexual maturity and sex ratio

Juvenile males and females begin to evolve sexual dimorphism features before they eventually reach sexual maturity. Sexual maturity was considered attained when the first couple was observed in copulatory amplexus. The first amplexus happened on 24/07/2014. *P. hawaiensis* attained sexual maturity within 45 days. Upon reaching maturity, individuals' size ranged on females from 3.18 mm to 5.269 mm and on males from 3.58 mm to 5,58 mm. This clearly indicates that the length increment per moult was higher in males compared to females, evidencing males grew faster than females. Since not all individuals could be correctly sexed during the first periods of the experiment, sex ratio of the cohort was achieved based on the number of females and males at the point of their sexual maturity. Total number of females: 46; males 27 presenting a sex-ratio of 63,01% females to males. Sexual maturity was identified for all cohort individuals at September 7th 2014, as the cohort completed 2 months.

Cephalothorax and full body measures

In amphipods, correlating size between cephalothorax and full body size lengths is a common practice in order to reduce data fault, due to the fact that the last appendeges (abdomen) may be hidden or curved.

Figure 8 indicates the correlation between cephalothorax and total body size at different classes (determined in months) from June 6th 2014 until October 21st 2015. It is possible to observe that from November 2014 on that the actual size of female individuals do not seem to have any real growth.

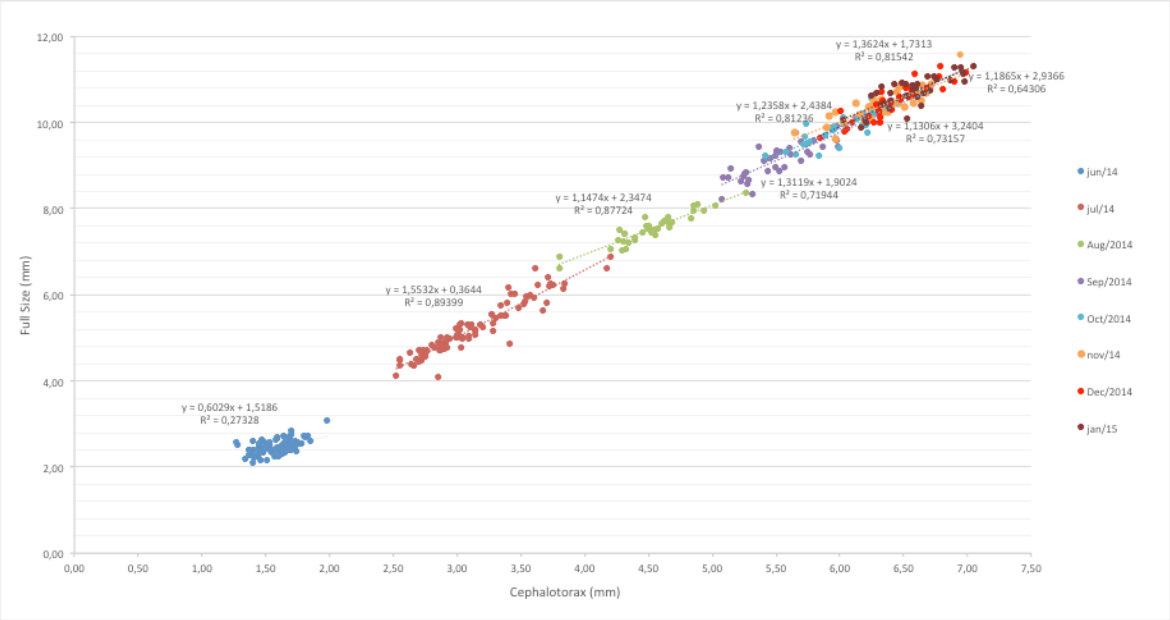

Figure 8. Size measurement correlation between cephalothorax size (x axis) and full body size (y axis) in millimeters for the initial 8 months following a cohort of *Parhyale hawaiensis.*

The correlation value obtained for *P. hawaiensis* was of R^2 = 0, 9948 and is represented by the linear equation obtained from individual measurements of the evaluated cohort (Figure 9). In comparison, figure 8 also shows the equation obtained for the wild population by Alegretti 2015.

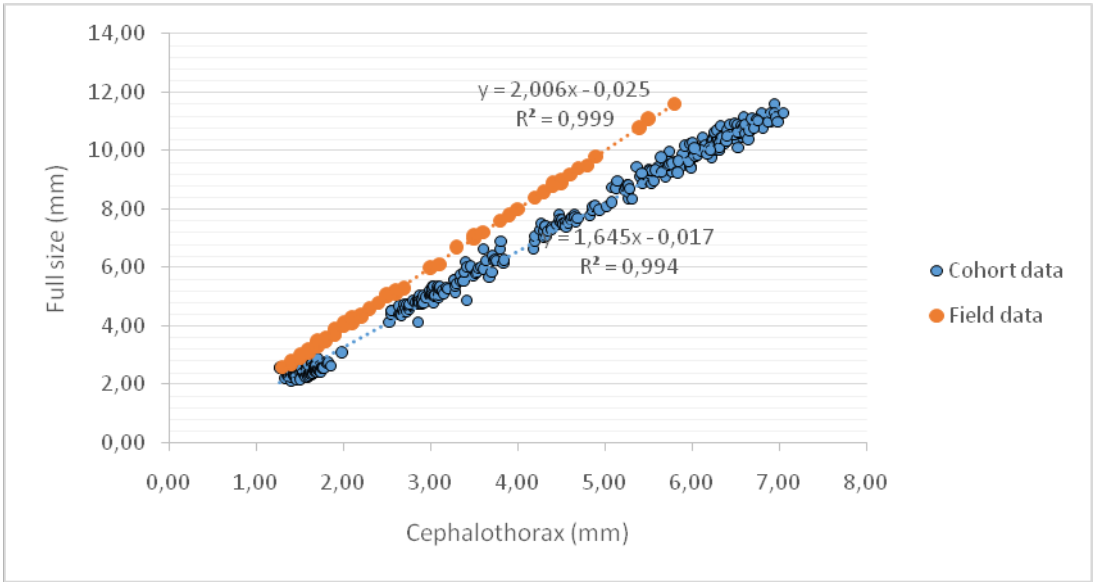

Figure 8. Size measurement correlation between cephalothorax (x axis) and full body size (y axis) in millimeters of the *P. hawaiensis* cohort maintained in laboratory culture (present work data) and wild data obtained by Alegretti (2015).

The Cohort and Time-specific Life Table

Monthly intervals were selected to represent the classes for the construction of the time-specific life table. The size of the cephalothorax of all the female individuals of the cohort was measured, presenting, up to date, 17 different classes.

Starting with 116 juveniles already allocated in a container together, on 06 of June of 2014, the cohort was maintained until the date of December 19th, 2015. Due to the end of the master program period, the final part of the time-specific life table was interrupted. After observing the decay of the cohort for 17 months, it can be hypothesized that its life span is of 24 months. For the projected time we considered a constant mortality, dividing it between the remaining months as suggested by Dr. Antonio Nogueira from Aveiro University, Portugal (personal communication February, 2015).

Table 3 presents the cohort decay, by counting the number of individuals remaining at the selected dates for culture manipulation and individual body size measurements.

Table 3. Number of individuals alive from the cohort at the dates designated for the manipulation, count and measurement of individuals.

Date	Number of Individuals
06/06/2014	116
02/07/2014	91
07/08/2014	73
04/09/2014	67
02/10/2014	66
06/11/2014	66
04/12/2014	65
08/01/2015	63
06/02/2015	62
05/03/2015	61

01/04/2015	61
01/05/2015	59
03/06/2015	59
08/07/2015	57
06/08/2015	54
10/09/2015	54
21/10/2015	52

To create comparative grounds, the time-specific life table constructed on this work and the age-specific life table created by Alegretti (2015), mean cephalothorax measures were adapted to monthly age classes (Table 4). Unfortunately, photographs taken in May and June 2015 was corrupted so that data over lengths measurements were not possible for this period.

Table 4. Average size of cephalothorax of the individuals measured on the established dates of manipulation and cohort monitoring.

Date	Cefalotórax (mm)	Standard Deviation
Jun/2014	1,6	0,14
Jul/2014	3,1	0,38
Aug/2014	4,5	0,29
Sep/2014	5,5	0,27
Oct/2014	6,1	0,32
Nov/2014	6,3	0,27
Dec/2014	6,4	0,29
Jan/2015	6,6	0,26
Feb/2015	6,75	0,32
Mar/2015	6,89	0,22
Apr/2015	6,971	0,18
May/2015	Data corrupted	Data corrupted
Jun/2015	Data corrupted	Data corrupted
Jul/2015	7,1	0,24
Aug/2015	7,2	0,29
Sep/2015	7,3	0,30
Oct/2015	7,2	0,25

The growth size of the cohort individuals, using the cephalothorax measure and its respective standard deviation, is represented graphically through time (Figure 10). As a comparative analysis, the blue points represent sizes obtained from the cohort in laboratory conditions, while the orange points represent sizes for wild individuals obtained by Alegretti (2015). It seems to indicate that in lab individuals' growth is more rapid at the beginning of the development in relation to wild individuals. Around the 8th month of life, growth almost stops in lab populations. Probably, the same phenomenon occurs in wild populations too, but with smaller sizes.

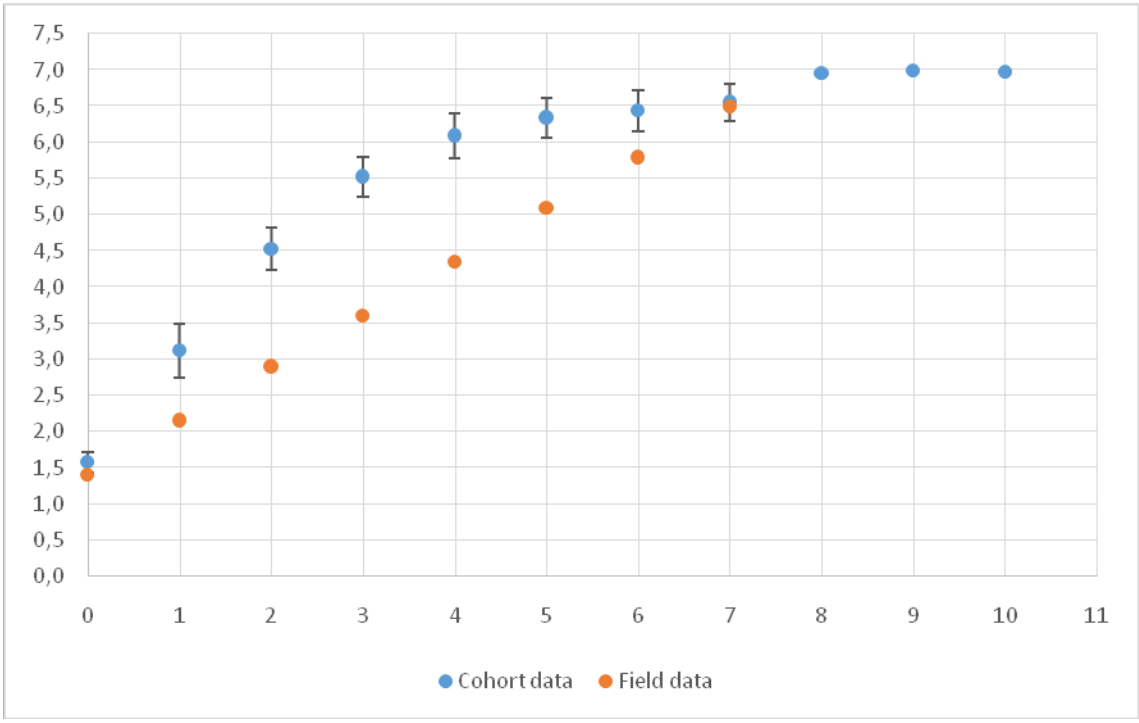

Figure 10. Average growth of individuals obtained in measurements and standard deviation in the cohort for each class in blue. In orange, values of size for each class estimated for and from wild data obtained by Alegretti (2015). Time (x axis) considered is monthly and growth (y axis) is presented in millimeters.

Fecundity (mx) was obtained by dividing the total number of juveniles born in each class by the total number of ovigerous female at the corresponding class (Table 5). Female individuals in classes 0 and 1 are juveniles before reaching maturity, therefore fecundity for both these classes is equal to 0 and are irrelevant for the calculation of fecundity.

Table 5. Months, classes, number of females per class, number of juveniles and fecundity (mx) for each class. Proj. = projected.

X (Months)	Classes	Number of Females	Number of Juveniles	mx
June/2014	**0**	77	0	**0,00**
July/2014	**1**	61	0	**0,00**
August/2014	**2**	49	31	**0,63**
September/2014	**3**	45	134	**2,98**
October/2014	**4**	44	237	**5,39**
November/2014	**5**	44	250	**5,68**
December/2014	**6**	43	278	**6,47**
January/2015	**7**	42	280	**6,67**
February/2015	**8**	41	303	**7,39**
March/2015	**9**	41	328	**8,00**
April/2015	**10**	41	279	**6,80**
May/2015	**11**	39	258	**6,62**
June/2015	**12**	39	290	**7,44**
July/2015	**13**	38	262	**6,89**
August/2015	**14**	36	274	**7,61**
September/2015	**15**	36	297	**8,25**
October/2015	**16**	35	270	**7,71**
Projected Nov./2015	**17**	**30**	**201**	**6,70**
Projected Dec./2015	**18**	**25**	**180**	**7,20**
Projected Jan./2016	**19**	**20**	**150**	**7,50**
Projected Feb./2016	**20**	**15**	**100**	**6,67**
Projected Mar./2016	**21**	**10**	**70**	**7,00**
Projected April/2016	**22**	**5**	**30**	**6,00**
Projected May/2016	**23**	**0**	**0**	**0,00**

In order to identify the correlation between sizes of the females, in this case already divided into the different established classes, and the number of eggs they carry in their marsupial pouch, a specific sub sampling was considered. 20 females were randomly selected from LEAL *P. hawaiensis* cultures. Figure 11 presents the fecundity correlation between female size and number of eggs, along with the equation, the linear trend line and value of R^2.

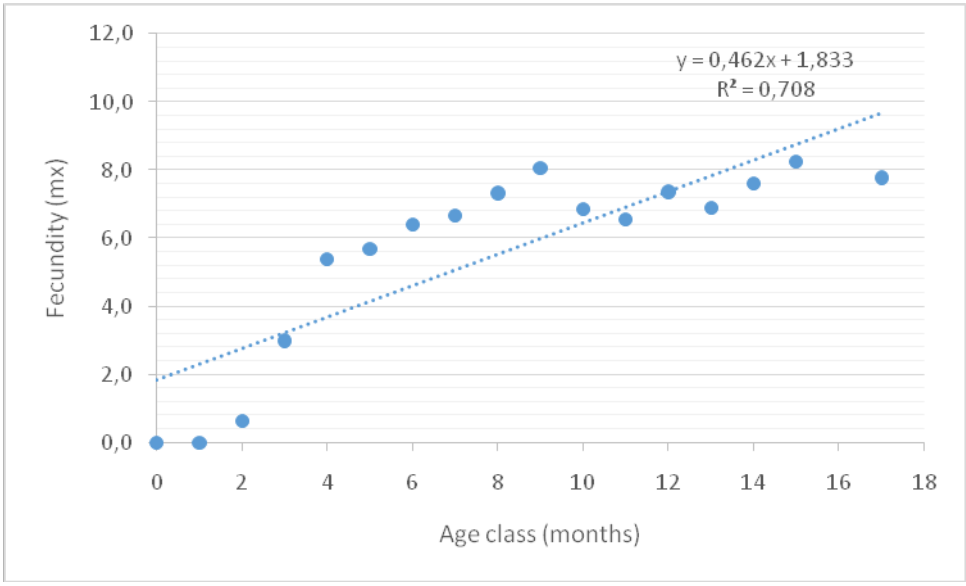

Figure 11. Tendency line, equation and R^2 correlation value for the female body size (x axis) divided by months and number of eggs (y axis) in their marsupial pouch.

Life and fecundity table extracted from the cohort follow up are presented in Table 6. The first column, denominated "x" indicates time and it refers specifically to the classes divided by months. The second column indicates the class. After inferring time intervals for each class and standardized size, the respective columns follow as *Nx*, *Sx*, *lx*, *Dx* and *qx* respectively representing in each class, the number of female individuals (initially defaulted to 1000), the number of surviving, the survival rate, the number of deaths and the death rate (GOTELLI, 2007).

Table 6. Life table constructed by cohort follow up. *Nx*: number of female individuals (extrapolated to 1000) present in each class; *Sx*: proportion of survivors between the respective class and the previous class; lx: proportion of survivors of the respective class in relation to the initial (*N0*) class; *Dx*: number of deaths for the respective class in comparison to the previous class; *qx*: probability of death between the respective class and the previous one to it; *mx*: mean number of juveniles produced per female for the corresponding class.

X (Months)	Classes	Time-specific Life Table					Fecundity Table		
		Nx	Sx	lx	Dx	qx	mx	lx*mx	lx*mx*X
June/2014	**0**	1000	0,78	**1,00**	216	0,216	0	0,0	0,00
July/2014	**1**	784	0,80	**0,78**	155	0,155	0	0,0	0,00

August/2014	**2**	629	0,92	**0,63**	52	0,052	31	0,6	0,40
September/2014	**3**	578	0,99	**0,58**	9	0,009	134	3,0	1,73
October/2014	**4**	569	1,00	**0,57**	0	0,000	237	5,4	3,06
November/2014	**5**	569	0,98	**0,57**	9	0,009	250	5,7	3,23
December/2014	**6**	560	0,97	**0,56**	17	0,017	278	6,4	3,59
January/2015	**7**	543	0,98	**0,54**	9	0,009	280	6,7	3,62
February/2015	**8**	534	0,98	**0,53**	9	0,009	303	7,3	3,92
March/2015	**9**	526	1,00	**0,53**	0	0,000	328	8,1	4,24
April/2015	**10**	526	0,97	**0,53**	17	0,017	279	6,9	3,61
May/2015	**11**	509	1,00	**0,51**	0	0,000	258	6,6	3,33
June/2015	**12**	509	0,97	**0,51**	17	0,017	290	7,4	3,75
July/2015	**13**	491	0,95	**0,49**	26	0,026	262	6,9	3,39
August/2015	**14**	466	1,00	**0,47**	0	0,000	274	7,6	3,54
September/2015	**15**	466	0,96	**0,47**	17	0,017	297	8,2	3,84
October/2015	**16**	448	0,86	**0,45**	65	0,065	270	7,8	3,49
Projected Nov/2015	**17**	**383**	**0,83**	**0,38**	**65**	**0,065**	**301**	**10,2**	**3,89**
Projected Dec/2015	**18**	**318**	**0,80**	**0,32**	**65**	**0,065**	**261**	**10,6**	**3,38**
Projected Jan/2016	**19**	**253**	**0,74**	**0,25**	**65**	**0,065**	**217**	**11,1**	**2,81**
Projected Feb/2016	**20**	**188**	**0,65**	**0,19**	**65**	**0,065**	**168**	**11,5**	**2,17**
Projected Mar/2016	**21**	**123**	**0,47**	**0,12**	**65**	**0,065**	**114**	**12,0**	**1,48**
Projected April/2016	**22**	**58**	**0,00**	**0,06**	**58**	**0,058**	**56**	**12,5**	**0,73**
Projected May/2016	**23**	**0**	**0,00**	**0,00**	**0**	**0,000**	**0**	**12,9**	**0,00**

Values calculated for net reproductive rate (R_0), generation time (T) and intrinsic rate of population growth (*r*) were calculated as shown on Table 7 comparing laboratory data with wild population values. Net reproductive rate and generation time values did not associate very well whereas intrinsic rate of population growth values are very similar.

Table 7. Values for reproductive potential (RO), generation time (T) and intrinsic rate of population growth (*r*) extracted from the cohort monitoring.

Demographic Parameters	Laboratory Values	Max Wild Values Alegretti (2015)
Net Reproductive Rate (R_0)	63,2 youngs per female	2,85 youngs per female
Generation Time (T)	12,1months	4,54 months
Intrinsic Rate of Population Growth (*r*)	0,34 per capita per month	0,27 per capita per month

Carrying Capacity & Logistic Curve

Based on the experiment created to validate population growth, data was derived from the maintenance of 10 initial parental couples and the subsequent generations, until the eventual stabilization of the originated population. This experiment lasted for 6 months. During each month, the number of adults (Table 8) and sex ratio were measured.

Table 8. Population growth experiment, evaluated monthly number of adult individuals.

Time (months)	Number of Individuals
0	20
1	102
2	244
3	511
4	565
5	570
6	558

Population growth experiment following 6 months of evaluation, behaved as expected presenting an initial exponential growth, an inflection of the curve and stabilization, creating a logistic curve as shown in Figure 12. The initial exponential population growth is attributed to a completely controlled laboratorial environment that eliminates the existence of predators and constant food supply. The curve

inflection at the third and the fourth month and subsequent population size stability is attributed to the fact that we have reached a physical barrier called carrying capacity (K). This means that every 2,5L container supports about 560-570 adult individuals.

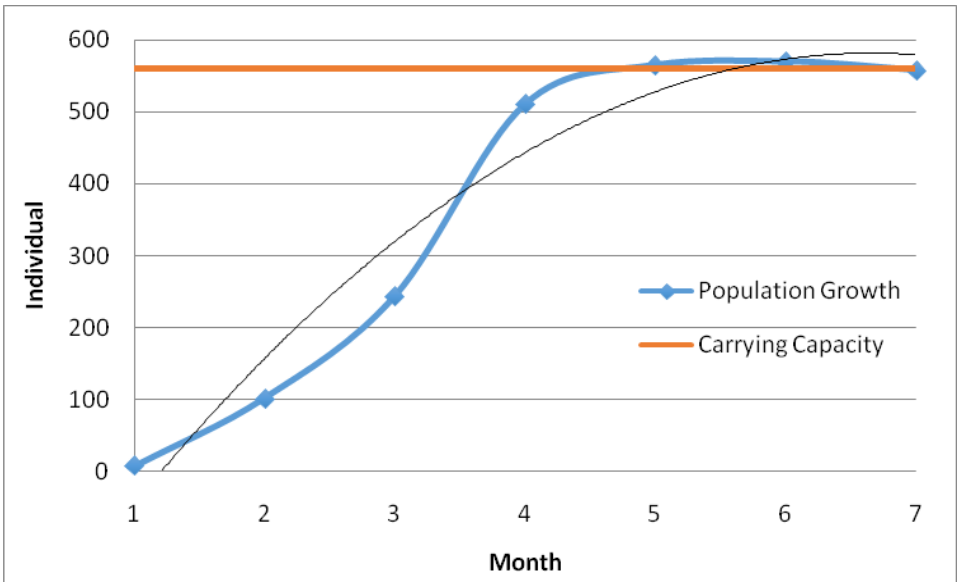

Figure 12. Population growth, the logistic curve and carrying capacity elaborated over the months (x axis) and number of individuals (y axis).

During this experiment, when population reached 570 individuals, the culture did not maintain the same environmental quality, considering the application of the maintenance as described earlier. Therefore, by this experiment we can infer that the carrying capacity at the laboratory is about 228 individuals per liter of reconstructed salt water, correspondent to the 570 individuals in the 2,5L container.

Information on the exponential growth of the population extracted from this experiment is based on the calculation of the instantaneous growth rate (*ri*), obtained for each time interval in months. It is possible to observe that the *ri* values is higher at the begging of the experiment and decreasing as the curve tends to stabilize (Table 9). Comparing the *ri* obtained from this experiment to *r* (*r* = 0,34) extracted from the life table construction, it is possible to infer that their

values are equivalent on the third month of the population growth experiment (ri = 0,34).

Table 9. Instantaneous growth rate (*r*) obtained for a population of *P. hawaiensis* in different time intervals and the mean rate.

Time (Months)	Instantaneous growth rate (*ri*)
1	0,636
2	0,427
3	0,346
4	0,266
5	0,213
6	0,176

Carrying capacity achieved in laboratory culture has been compared and considered to be very close to the one established by Alegretti (2015), K = 1230 number of individuals per 100g of algae. For comparative reasons, rescaling laboratory carrying capacity to a 5L container, K = 1140. It is also possible to infer and predict the cohort logistic curve based on intrinsic rate of population growth (*r*) values obtained from fecundity elaborated from the cohort follow up and life table construction and from Alegretti 2015 monthly measures (Figure 13).

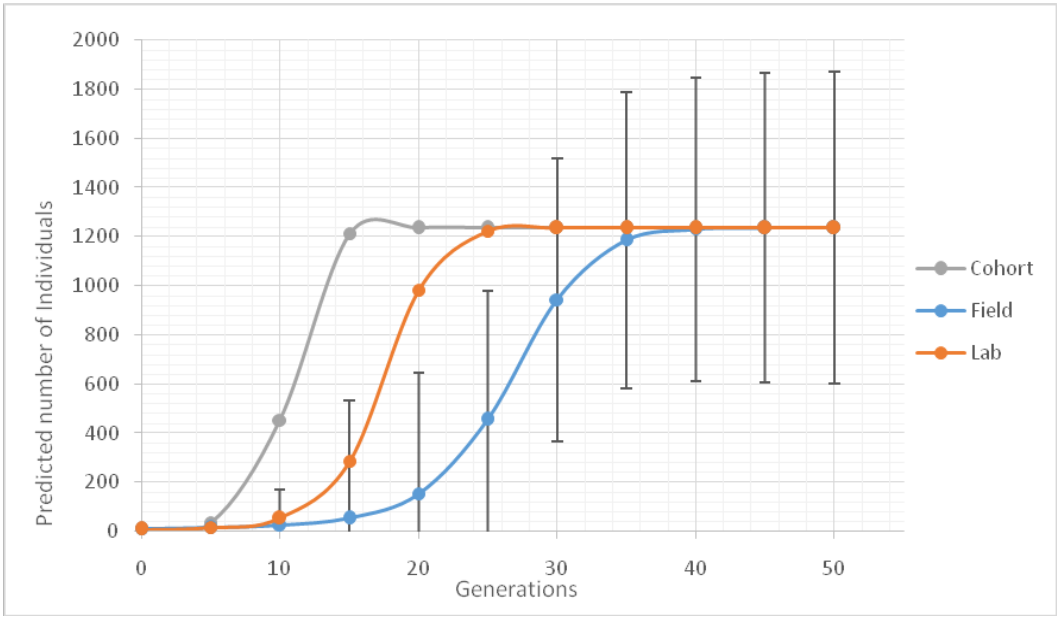

Figure 13. Model with three logistic curves obtained from cohort follow up, wild population (field) and lab population elaborated by number of generations (x axis) and number of individuals predicted (y axis).

DISCUSSION

Life history strategies are closely related to environmental conditions. Biotic and abiotic factors play a major role in conditioning several traits such as growth rates, sexual maturity, fecundity, survivorship, size, and morphometric differences (LAUDIEN, 1973; RUMBOLD et al., 2014). The energetic trade-off between these physiological needs have consequences on a particular species' life history. Physiological adaptation to altered environmental conditions requires energetic costs that may compromise other physiological needs, such as growth and reproductive success, both considered important Darwinian fitness traits for species' survival (DOYLE & HUNTE, 1981). Therefore, significant impairment of growth and reproduction will lead to a decline in natural populations, thus threatening species' survival and genetic diversity (WU & OR, 2005).

P. hawaiensis, considering wild or lab populations, reproduced year-round. Females produced broods consecutively (Iteroparity), exhibiting a multivoltine life cycle. Laboratory *P. hawaiensis* population compared to wild population, exhibits a delayed reproduction, higher fecundity associated with a slightly larger body size, Also experiences lower mortality and has a longer generation time; has long-lived, 20 months and is estimated to live up to 25 months, and appears to experience senescence, representing a classical K selection. In contrast, the wild population exhibits early reproduction, low fecundity associated with smaller body size. Also has a shorter generation time; and has short-lived, an estimated 8-12 months, representing a classical r selection (see ALEGRETTI, 2015). However, it must be noted that reliable information on potential life spans of amphipods is still scarce due to the fact that most investigations are based on wild samplings and therefore life spans is calculated in the presence of predation and other environmental constraints (BEERMAN & PURZ, 2013).

Although there are some theoretical, logical, and empirical difficulties associated with the r/K explanatory life history paradigm, this model is still applicable for making adaptive sense on the patterns of co-variation that exist among demographic variables. Such co-variation has been attributed to variation in adult body size, thought to be the target of selection (CHISHOLM et al., 1993).

Aravind et al (1984) emphasized the importance of phylogenetic constraints and concluded that there is no simple optimum life-history strategy, but that a combination of different life-history traits may be equally successful in a given environment. That opinion was confirmed by Sainte-Marie (1991) who suggested that phylogenetic and physiological constraints should be best considered for the interpretation of gammaridean life-history patterns.

The influence of environmental factors, related to the very nature of the habitat should not be discarded, such as food availability and predation pressure (STEARNS, 2000). Food resources are one of the main factors involved in growth and sexual maturation of crustaceans (CASTIGLIONI et al., 2007; CUZON et al., 2008). It has been suggested that in populations of some crustaceans (e.g. crabs, shrimps and amphipods) that reach adulthood at relatively smaller sizes, predation pressure is higher on larger organisms. Thus allowing it to reach maturity at younger age, and consequently at a smaller size, and to reproduce before being killed (RUMBOLD et al., 2015). This hypothesis implies that natural factors or pollutant concentrations may explain the increase in the presence of the phenotype small size in wild populations (WELLBORN 1994; SCHLINING & SPRATT 2000; ZHANG et al., 2004; RUMBOLD et al., 2015).

In laboratory, the apparently longer life span is coupled with a larger number of moults. However, the much larger life span is not directly related to individual's size, after individuals attained approximately 7 mm. From this size on it seems that moulting in female is related exclusively to reproduction. These findings may be explained by a particular reproductive pattern particular to related amphipod crustacean *Jassa slatteryi* (REHM et al., 2009). Males and females mature at about the same age. Adult females continue to moult but not to grow throughout their life. In contrast, males are sexually active with a terminal moult (NAIR & ANGER, 1979).

The process of moulting is essential for brood production in amphipods, as oviposition only can take place immediately after ecdysis. Shorter intermoult periods result in a quicker succession of broods, promoting a faster population growth (BEERMAN & PURZ, 2013). Under laboratory conditions, intermoult periods of adult crustaceans usually become longer over time, related to age

and/or size of the individual (CONAN, 1985). Nevertheless, increasing span of moult intervals following age progression does not obligatory result on growth rates decrease, as growth and moulting are not strictly coupled in amphipod crustaceans (HIGHSMITH & COYLE, 1991).

The cultivation in laboratory of *P. hawaiensis* has raised the net reproduction rate R_0 from 2,85 to 63,2, the time generation from 4,54 to 12,1 months and the maximum intrinsic rate of increase *r* from 0,27 to 0,34 (calculation based on a lifespan truncated in 25 months). Similar differences between polluted and pristine environments were detected in another peracarid, the tanaidacean *Apseudopsis latreillii* (de la OSSA CARRETERO et al., 2010), regarding chemical parameters. Oxygen, for instance, influences size in amphipod species (NEBEKER et al., 1992; CHAPELLE & PECK, 2004). Also, higher organic matter content could favor maturity at smaller sizes, as detected in several peracarid populations, when analyzed under stressful conditions, because they invest more energy on reproduction than on growth (CLARKE, 1987).

The stressful conditions of an environment may involve in resource shifting on reproduction and survival (STEARNS, 2000), which could explain the observed variations. Laboratory larger sizes would favor an increase in fecundity and fitness in females (RUMBOLD et al., 2012). In peracarid, fecundity can be affected by many environmental factors, such as pollutants, food availability, latitude, salinity and temperature (COREY, 1981; FRANCE, 1992; MARANHÃO & MARQUES, 2003; FORD et al., 2004; PENNAFIRME & SOARES-GOMMES, 2009).

Population-level effects, resultant from reproductive abnormalities as consequence of chemical exposure, are notoriously difficult to quantify from studies on wild populations due to the inherent complexities of the ecosystem with multiple and concurrent influences upon them. Appropriate laboratory tests, together with modeling approaches, offer the potential to develop tractable testing systems for assessing latent population-level consequences of contaminant exposure. In particular, laboratory tests that quantify reproductive success are necessary since these endpoints have population relevance (PAULL et al., 2008).

Several measures of fitness are available (TULJUPURKAR, 1990; ROFF, 1992; STEARNS, 1992; CAREY, 1993; KOZLOWSKI, 1993; CHARLESWORTH,

1994), but intrinsic rate of increase *r* and net reproductive rate R_0 are by far the two most commonly used ones. The intrinsic rate of increase is the rate of population increase in a closed population, assuming constant age-specific schedules of death and reproduction and a stable age distribution. Whereas the net reproductive rate is the average number of female offspring born to a female over her lifetime, again assuming constant age-specific schedules of death and reproduction (CAREY, 1993). Both measures estimate population growth rates, but *r* is scaled to time, whereas R_0 is scaled per generation and is independent of time (HUEY & BERRIGAN, 2001). Evolutionary ecologists (TRAVIS & HENRICH, 1986; ROFF, 1992; STEARNS, 1992; KAWECKI & STEARNS, 1993; KOZLOWSKI, 1993; BERRIGAN & KOELLA, 1994; CHARLESWORTH, 1994) have warned that these classical fitness measures, *r* and R_0, are not interchangeable and, consequently, the choice between measures must be guided by basic demographic context of the population at hand.

Net reproductive rate (R_0) maybe considered a good measure of population fitness, but this assumption is risky and, comparatively, population growth rate (*r*) is a much more secure index to population fitness (LEVIN et al., 1996). The rationale behind this is that R_0 measures only expected reproductive output ignoring it's timing inside the life cycle. The reproductive potential obtained from the lab population was much higher than those obtained from the wild population. Furthermore, *r* is inversely and strongly related to generation time, whereas R_0 is independent of generation time (COLE, 1954; LEWONTIN, 1965). Consequently, the shortening of generation time will cause increase in *r,* but will not affect R_0. Development time generally has a much larger impact on *r* than lifetime fecundity, at least when analyses are conducted on life-table data gathered at a single temperature (COLE, 1954; LEWONTIN, 1965; TRAVIS & HENRICH, 1986; STEARNS & KAWECKI, 1994). Generation time (*T*) in species with continuous reproduction such as *P. hawaiensis* is a rather deceptive concept, because it is represented by the mean age of the parental individuals of a whole offspring produced (generated) from one cohort. Therefore, a population with a faster growth rate exhibits a shorter generation time (CAUGHLEY, 1977).

The characterization of test organism's demographic responses to environmental factors is required for a correct interpretation of ecotoxicological

tests and for the definition of comparative endpoints and strategies for chronic and long-term toxicity testing (NEUPARTH et al., 2002). It seems that in the laboratorial environment with constant culture conditions and low population densities, despite rapid growth, the intrinsic rate of increase is probably the most reasonable single measure of absolute population fitness. Even though calculated only for females and dependent on the notion of a constant age distribution.

Although any variation in environmental parameters greatly complicates the description of the life history of a multivoltine species, as *P. hawaiensis* (SINERVO & DOYLE, 1990), the intrinsic rate of increase *r* seems to provide a reasonably good comparison of absolute fitness in test exposure, since its values are approximately the same for both, wild and cultivated populations.

Once cultivation in a controlled environment begins, another difficulty appears. Genetic changes in the fitness of cultivated populations are almost inescapable. Doyle and Hunte (1981) termed these changes as “domestication”, a usage that is consistent with the ordinary meaning of a word that denotes both the shielding of an animal from unfavorable environmental conditions and long-term genetic adaptation to an artificial environment. In experimental physiology and ecology, domestication may result in an ever-increasing divergence between laboratory stocks and the ancestral populations in the world outside the laboratory. Information about the genetics of changes in fitness is urgently needed when a species is brought under cultivation.

In addition, phenotypic plasticity, the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behavior in response to environmental conditions, must be considered. Phenotypic plasticity generated, where life-history traits are passively responding to environmental variables (e.g. temperature), has been termed phenotypic modulation. Another form of environmentally induced phenotypic plasticity, developmental conversion, is generated in response to environmental cues such as temperature or photoperiod (SMITH-GILL, 1983). This response usually represents adaptation to seasonal deterioration of environments. Both forms of environmentally induced phenotypic plasticity are important to consider when comparing the life-history

strategies parameters in laboratory and wild populations (SINERVO & DOYLE, 1990).

For many ecotoxicologists, the choice of endpoint between *r* versus R_0 is academic, as few will have access to (or find it practical to generate) life table data. In this matter, *ri* (instantaneous rate of growth) has been appointed as an alternative endpoint to evaluate the population effects (lethal and sub lethal) of contaminants (STARK; BANKS, 2003). Values of *ri* are based on monitoring the growth of a population for a predetermined time. Time, frequently shorter than the necessary time to obtain *r*. Differently of what occurs when following a cohort, the calculation of *ri* is basically made from the initial number and final number of individuals of a whole population (even with generation overlap) along the chosen time interval.

The calculation of these two growth rates, the intrinsic (*r*) and the instantaneous (*ri*), generates, as each particular result, a unique number. An important concern in order to establish this comparison viable is the determination of the time interval set for the monitored population for the obtainment of *ri* to be compared to a *r* reference value, extracted from the life table. Duration of ecotoxicological tests, particularly chronic tests, may be a major technical problem. Ideally, a chronic test should comprise the entire organism life cycle, i.e. from birth to progeny production, or at least the most sensitive stages (HILL et al., 1994). Although for scientific purposes long-term tests may be of great utility, routine application of such tests would not be practical and cost-effective. A compromise of 4 weeks (28 days) has been used namely for chronic sediment toxicity tests with amphipods and other invertebrates (NIPPER & ROPER, 1995; MARTINEZ-MADRID et al., 1999). Here, the adequate time for obtaining a *ri* compatible to *r* would surpass the indicated 4 weeks period to double that time, 8 weeks.

CONCLUSION

In conclusion, demographic endpoints such as instantaneous growth rate (ri), net reproductive rate (R_0) and generation time (T) don't appear to be effective for lab populations of *Parhyale hawaiensis*. Perhaps parameters such as survival rate (l_x), fecundity (m_x), sexual maturity achievement size and carrying capacity (K) could be more efficient endpoints, when coupled to the intrinsic growth rate (*r*).

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