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# Application of a gelatinous zooplankton tank for the mass production of larval Caribbean spiny lobster, *Panulirus argus*

Jason S. Goldstein[1,a] and Brian Nelson[2]

[1] Center for Marine Biology and Department of Biological Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824, USA
[2] New England Aquarium, Fishes Department, 1 Central Wharf, Boston, MA 02110, USA

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**Abstract –** Successful commercial aquaculture production of spiny lobster is, for the most part, still constrained by an array challenges including the development of nutritionally complete and cost-effective feeds, control of disease vectors, and the design of larval mass culture tanks. Culture tank designs for larval production are a critical step to facilitating the most favorable combinations of water flow, food contact, and larval survivorship over the course of development. The evolution of new plankton-kreisels that are used by aquariums to culture and exhibit gelatinous zooplankton (e.g., jellyfish) provide a unique opportunity for testing the feasibility for spiny lobster larval culture, particularly with tropical species such as Caribbean spiny lobster (*Panulirus argus)* whose larval duration, although complex, is comparatively shorter than other spiny lobsters. Here, we report on the feasibility of culturing *P. argus* larvae (i.e., phyllosoma) from hatch to Stage VI using large (180 L) modified acrylic plankton-kreisels. We compared overall growth and survival of phyllosoma at starting densities of 5000 (~27.8 larvae $L^{-1}$) and 2500 individuals (~13.8 larvae $L^{-1}$) and found no significant difference with respect to survival or mortality through to 65 days ($\chi^2 = 1.595$; $df = 1$; $p = 0.2066$) resulting in mean survival rates of 60.7% (s.e. = ±3.7) and 54.5% (s.e. = ±3.2), respectively. Comparable growth was also achieved between both densities to Stages V and VI (mean body lengths of 7.5 and 10.2 mm, respectively) at 25.1 ± 0.41 °C and pH = 8.1. Phyllosoma utilized the entire tank volume and displayed minimal entanglement. The application of such tank designs for larval spiny lobster culture not only contributes to future designs for aquaculture production, but also provides a useful platform for conducting behavioral studies for this complex larval phase.

**Key words:** *Panulirus argus* / Spiny lobster / Phyllosoma / Kreisel / Aquaculture

## 1 Introduction

Successful commercial aquaculture production of spiny lobster is, for the most, part still constrained by an array of biological, economical, and technological challenges including the development of nutritionally complete and cost-effective feeds, control of disease vectors, and the design of flow-efficient mass culture tanks (Bourne et al. 2006; Matsuda and Takenouchi 2007). The Caribbean spiny lobster (*Panulirus argus*) is considered the most important commercial lobster in the western hemisphere due to its large socio-economic impact and disproportionate catch (46% world-wide; FAO 2009) compared with other lobsters. As a target of intense fishery exploitation, many populations are declining (Ehrhardt 2005; Chavez 2009) and, as a result, *P. argus* is considered a valid candidate for continued aquaculture development (see Jeffs and Davis 2003, for review). Despite recent successes in *P. argus* larval development, nutrition, and grow-out (Matthews and Maxwell 2007; Goldstein et al. 2008; Cox et al. 2008) substantial hurdles remain, including the successful rearing of large numbers of spiny lobster larvae (i.e., phyllosoma) through to settlement.

Culture tank designs for larval production are a critical step to facilitating the most favorable combinations of water flow, food contact, and larval survivorship throughout the course of development. A number of tank designs have been used in the rearing of marine invertebrate plankton with good success originating from the Greve plankton-kreisel used for at-sea collection vessels, to the more recent exhibitions of gelatinous zooplankton, particularly jellyfish and ctenophores, at public aquariums (Greve 1968; Hamner 1990; Raskoff et al. 2003). Tank designs and trials using modified plankton-kreisels for the culture of larval lobsters (e.g., American lobster, *Homarus americanus*) have demonstrated mixed results but have contributed valuable data on growth, larval duration, diet, and survival (Hughes et al. 1974; Illingworth et al. 1997; Kittaka 1997; Matsuda and Takenouchi 2005). The evolution of new plankton-kreisels that are used in the public sector by aquariums and zoos (see Sommer 1993)

[a] Corresponding author: j.goldstein@unh.edu

provides a unique opportunity for testing the feasibility for spiny lobster culture, particularly with species such as *P. argus* whose larval duration, although complex, is comparatively shorter (4–6 months) than other species (Goldstein et al. 2008). Additionally, the design and mechanics of plankton-kreisels make them hydrodynamicaly-suited to minimize larval mechanical stress and decrease larval aggregation and entanglement (Calado et al. 2003; Matsuda and Takenouchi 2005).

Here, we report on the feasibility of mass culturing early- and middle-stage *P. argus* larvae using modified plankton-kreisels that are proven designs for jellyfish culture. We compared overall growth and survival of phyllosoma of *P. argus* at two densities and discuss the benefits and limitations of using such a tank design for future work.

## 2 Materials and methods

### 2.1 Tank holding and hatching

A single female *P. argus* lobster (carapace length = 94 mm) with an intact spermatophore was collected by SCUBA divers off of the Bimini Islands, Bahamas (25.392 N; 79.197 W decimal lat.-longitude) and transported by air freight to the New England Aquarium (Boston, Massachusetts, USA) where all subsequent work was conducted. The adult lobster was held in a 2100 L fiberglass holding tank, equipped with an aragonite filter bed, UV- filtered seawater (22–23 °C, 33–35 psu), protein skimmer, and a full-spectrum 14:10 h light-dark regime. The lobster was fed daily with fresh squid or shrimp and extruded its egg mass 13 days later. After a gestation period of 45 days, successive batches of phyllosoma hatched over a two-day period and were carefully siphoned from the tank, equally mixed from both days, and added to the experimental kreisel tanks to minimize potential larval viability effects from early or late hatching events (see Wickins et al. 1995). A total of 5000 or 2500 phyllosoma were stocked into two duplicate 180 L kreisel tanks ($n$ = 4 total). Only strong swimming animals (assessed by phototactic behavior with illumination sticks) were used for these trials.

### 2.2 Culture tank and system design

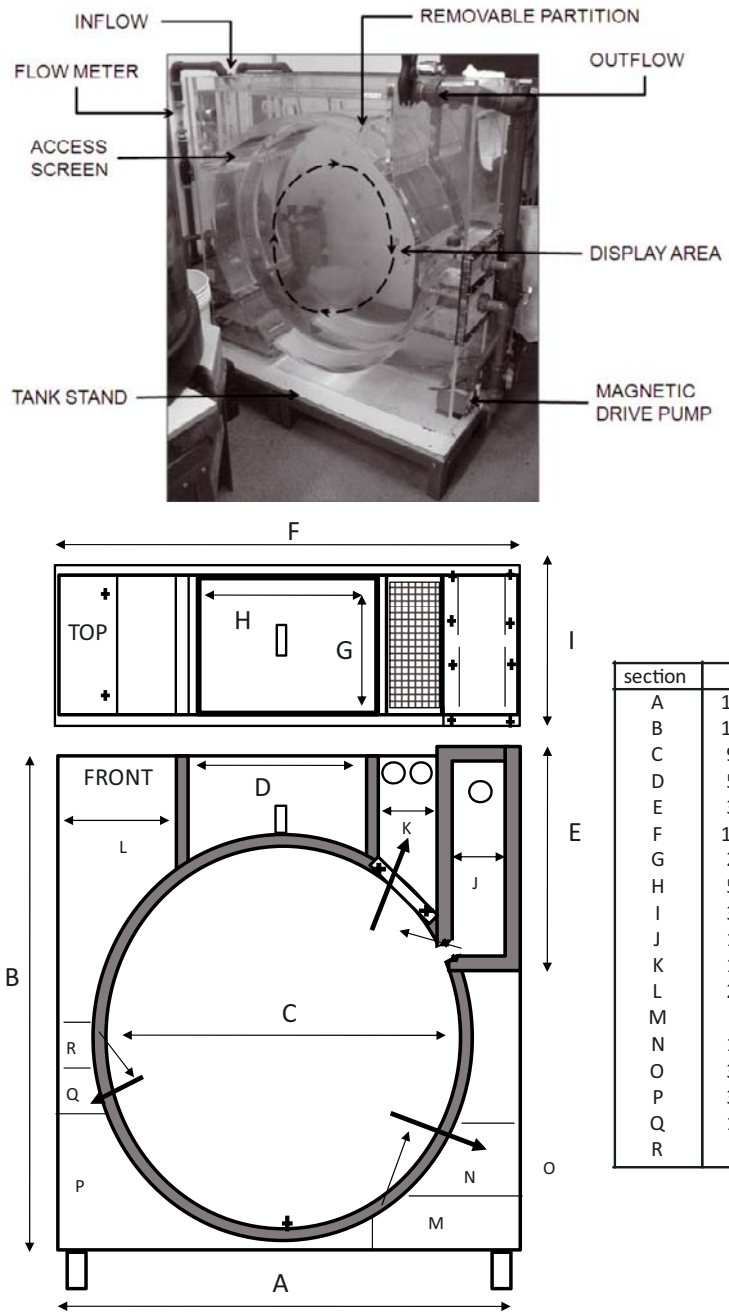
All tanks were housed in the Jellyfish Culture Lab at the New England Aquarium's Edgerton Research Laboratory, Boston, Massachusetts USA. Kreisel tanks had an effective working volume of 180 L (key dimensions: length = 122.1 cm, width = 32.0 cm, height = 122.1 cm, diameter = 96.0 cm; Fig. 1) and were constructed of clear acrylic (Envision Acrylics, Beverton, Oregon, USA). Water flow was facilitated by a magnetic drive pump with a flow rate of 5.6 L $min^{-1}$ (Iwaki America, Inc. Holliston, Massachusetts, USA; Fig. 1). Special features of this tank include a laminar downwelling flow (originating from the surface), curved sides and bottom, and the placement of fine-mesh screened partitions that separate the outflow of the tank, and the placement of large fine-mesh screened partitions that separate the drainage out of the tank, protecting animals and allowing for efficient cleaning (Fig. 1; also see Raskoff et al. 2003, for details). Overhead lighting was provided by a full spectrum 75 W metal halide fixture (Aquatic Ecosystems, Apopka, Florida, USA) on a 12:12 h light-dark cycle. Kreisel tanks were linked to a common fiberglass seawater reservoir containing biological (biowheel filter apparatus) and mechanical filtration to 0.2 $\mu$m, protein foam fractionation, and UV sterilization (Fig. 1). Water turnover for this system was calculated at ~75% of total volume per day and was sourced from instantaneous temperature-controlled seawater (30–35 psu) through a Hot Shot 72 K heater (Process Technologies, Mentor, Ohio, USA) preset to 25.0 °C. An additional 1000 W titanium immersion heater and digital thermostat controller (Cleveland Process Corp., Homestead, Florida) was mounted in the reservoir tank to insure a stable temperature regime for the system. Environmental parameters (temperature, salinity, and pH) were monitored and logged using a Sensaphone 1104 automated device (Phonetics, Inc., Aston, Pennsylvania, USA).

### 2.3 Feeding

Both tank densities were fed daily a combination of food items including finely minced mussel gonad (*Mytilus edulis*), jellyfish (*Aurelia aurita*), and live *Artemia salina* (ranging from nauplii to adults). Whole mussels were captured locally and kept for one-week in UV sterilized seawater (32–35 psu); mussel gonad was extracted daily from fresh animals and washed thoroughly in distilled water. *Artemia* (Grade A, San-Francisco Bay Brand, Newark, California, USA) were decapsulated using a bleach-sodium thiosulfate protocol and, upon hatch, were fed a liquid HUFA supplement at a density of 0.5 g $L^{-1}$ over a 16–20 h period prior to feed out (Selco®, Aquatic Ecosystems, Apopka, Florida, USA). Jellyfish (fed enriched *Artemia* nauplii) were selected from those that were being cultured in the same facility in conditions similar to those described for lobster larvae. Small jellyfish (5–8 cm bell diameter) were extracted daily from their tanks and roughly chopped except for the oral arms and tentacles, which were removed. From these preparations feed components were rationed (doubled for the higher density treatment) as follows: (1) *Artemia* at a density of 4–6 nauplii $ml^{-1}$ for early stages and 1–2 adults $ml^{-1}$ for middle stages, 3 times a day; (2) 150–200 pieces of mussel gonad chopped into small ~1 $mm^3$ cubes for early- and 200–205 pieces (4 $mm^3$) for middle-staged phyllosoma, once a day and; (3) 250 ml of chopped jellyfish for all stages, once a day. Feed amounts were modeled similarly to previous culture protocols (Kittaka 1997; Matsuda and Yamakawa 2000).

### 2.4 Husbandry and growth

Twice per day, uneaten food, larval molts, and mortalities were carefully siphoned (and counted) from each kreisel and the drainage screens removed and cleaned. Once every10 days, all phyllosoma were siphoned into temporary holding tanks (number of animals were then estimated volumetrically) and all kreisels were cleaned and washed with a 5% chlorine solution and refilled. A sub-sample ($n$ = 5/tank/week)




| section | cm |
|---|---|
| A | 122.1 |
| B | 122.1 |
| C | 96.0 |
| D | 58.0 |
| E | 38.0 |
| F | 122.1 |
| G | 25.0 |
| H | 58.0 |
| I | 32.0 |
| J | 11.0 |
| K | 15.0 |
| L | 26.7 |
| M | 7.6 |
| N | 11.4 |
| O | 31.8 |
| P | 38.1 |
| Q | 14.0 |
| R | 7.6 |

**Fig. 1.** **(a)** Photo of kreisel tank; **(b)** Plankton-kreisel design used for the culture of early- and middle-stage phyllosoma of *Panulirus argus*. Total tank volume is 180 L with key dimensions as follows: length = 122.1 cm, width = 32.0 cm, height = 122.1 cm, diameter = 96.0 cm. Tank is constructed of clear acrylic (3.2 cm thick) and features a laminar down-welling flow, curved sides and bottom, and the placement of large fine-mesh screened partitions that separate the drainage out of the tank, protecting animals and allow for efficient cleaning. Drawing adapted from online illustration at: http://www.mbari.org/midwater/tank/tank.htm.

of phyllosoma was removed and preserved in a 4% solution of formalin in sterile seawater then transferred to 70% ethanol vials for later staging. Specimens were later examined under an Olympus SZ-61 dissecting scope and staged according to methods described in Goldstein et al. (2008). When phyllosoma were observed with bacterial infections (whitening and necrosis of the digestive gland), we administered the antibiotic streptomycin into the tank culture water at a concentration of 10–15 mg $l^{-1}$. The tank was kept in a static mode (i.e. no incoming water flow) for 3–4 h however, we did provide moderate aeration to keep animals from sinking to the bottom. On three occasions (days 1, 15, and 35), microbial samples were collected from the kreisel tanks and cultured using standard methods by veterinary diagnostic staff (see techniques in Bolinches et al. 1988). Briefly, small (10 ml) samples were removed in sterile sample bottles along with a swab from inside each tank using a sterile brush. In addition, samples of mussel

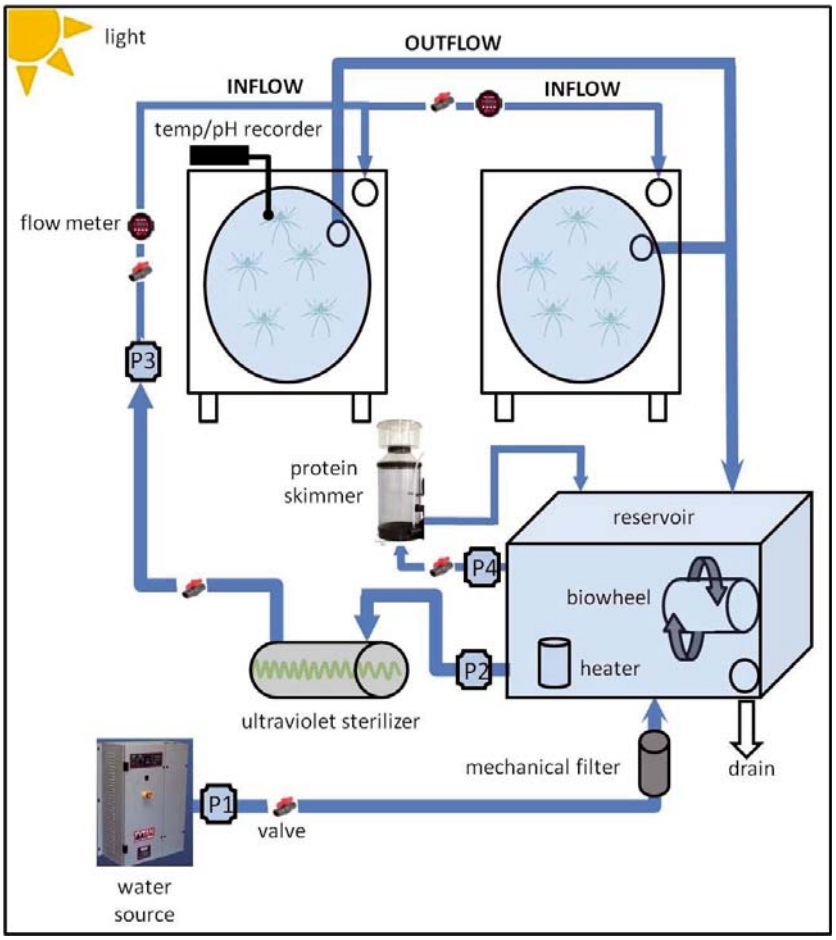



**Fig. 2.** Flow diagram for the design and operation of plankton kreisel tanks and their associated supporting components (e.g., pumps, P1, P2...) in a semi-closed seawater system. Water turnover for this system was calculated at ~75% of total volume per day and was sourced from instantaneous temperature-controlled seawater (25.0 °C) at a salinity of 30–35 psu. See text for component details.

gonad, *Artemia*, and jellyfish were tested at the beginning and end of the study.

### 2.5 Statistics

Phyllosoma densities were converted to percent survivors per day and the subsequent proportion of overall mortality with time. Densities were pooled and analyzed using the PROC LifeTest algorithm using SAS v. 9.2 (SAS Institute Inc., Cary, North Carolina, USA), where the response variable (survival time) was used to measure the duration of time until a specified event (mortality) occurred. Specifically, this algorithm is designed to compute nonparametric estimates of the survival distribution function using the Kaplan and Meier product limit comparing the survival curves of two or more groups (Sokal and Rohlf 1995).

## 3 Results

### 3.1 Phyllosoma survival

Overall, there were no significant differences with respect to survival or mortality for the two density treatments during 65 days of culture from hatch (DAH) using plankton kreisel tanks (SAS PROC LifeTest; $\chi^2 = 1.595$; $df = 1$; $p = 0.2066$; Fig. 2). A starting density of 5000 phyllosoma (27.8 animals $L^{-1}$) for each of two replicated tanks resulted in a mean survival of 60.7% (s.e. = ±3.7). Conversely, a density of 2500 phyllosoma (13.8 animals $L^{-1}$), yielded a mean survival rate of 54.5% (s.e. = ±3.2). Density levels precipitously dropped off 8.7% in the 5000 density treatment at the outset of a bacterial outbreak between days 39–46 with a total loss of 1625 animals compared with a 4.8% decrease, or 335 animals, for the 2500 tank density over the same time period (Fig. 2). Microbial identification indicated the presence of marine *Vibrio* sp. in all tanks, but highest in the larger tank densities. Large numbers of *Vibrio* sp. were also accounted for in jellyfish and phyllosoma alike, but lower among *Artemia* and mussel gonad.

### 3.2 Phyllosoma growth

Comparable growth was also achieved between both densities to Stages V and VI (mean body lengths = 7.5 mm and 10.5 mm, respectively) or ~9–10 instars at 25.1 ± 0.41 °C (pH = 8.1; salinity 32–35 psu) and was not significant

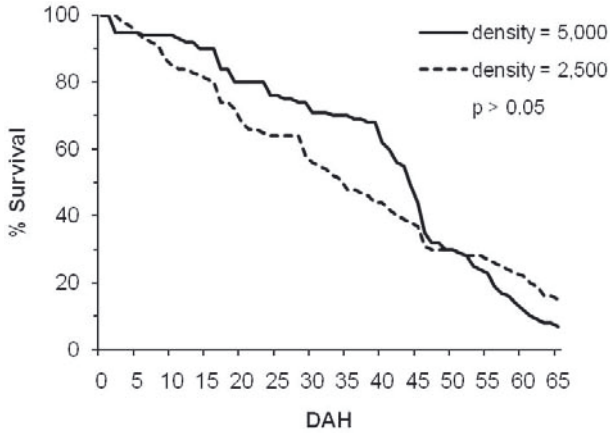



**Fig. 3.** Total % survivorship and mortality for batches of *P. argus* phyllosoma at two separate densities (2500 and 5000) over a 65-day cycle. There we no significant differences in either metric (SAS PROC LifeTest; $\chi^2 = 1.595$; $df = 1$; $p = 0.2066$). Density levels precipitously dropped off 8.7% in the 5000 density treatment at the outset of a bacterial outbreak between days 39–46 with a total loss of 1625 animals compared with a 4.8% decrease or 335 animals for the 2500 tank density over the same time period.

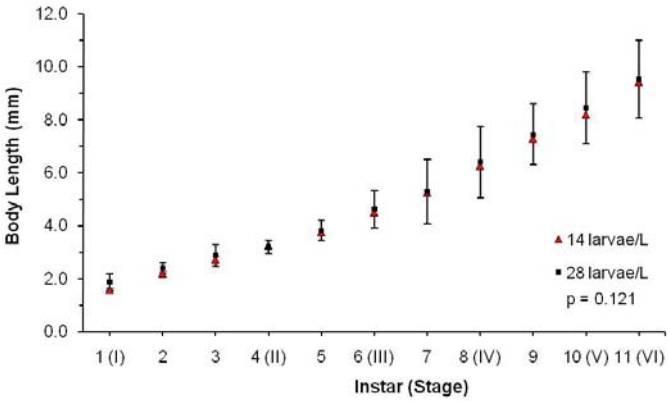



**Fig. 4.** Overall growth between densities of 2500 (14 larvae $L^{-1}$) and 5000 (28 larvae $L^{-1}$) Stages V and VI (mean body lengths = 7.5 mm and 10.5 mm, respectively) or ~9–10 instars; comparable growth was not significant (unpaired *t*-test; $t = 2.45$; $df = 120$; $p = 0.121$). Estimated for both densities, the ratio of Stage V:VI was ~1:2 using the described volumetric technique. Intermediate growth stages of phyllosoma (instars) are indicated with Stages in ( ).

(unpaired *t*-test; $t = 2.45$; $df = 120$; $p = 0.121$; Fig. 4). We estimate that for both densities, the ratio of Stage V:VI was ~1:2 using the described volumetric technique.

Over the course of the study, we also made several noticeable observations related to the use of tank space by phyllosoma including a marked transition from the upper 2/3 of the tank (1–45 DAH) to utilizing more of the bottom 1/3 of the kreisel towards the end of the study (>50 DAH). Most of the molting that occurred was synchronous (within a few days) between instars and stages and molts typically collected at the surface of the kreisel in the early morning. Molts were routinely collected on the screened partitions where they were readily removable. In both densities, we noticed a minimal amount of larval entanglement throughout the entire trial period.

## 4 Discussion

Kittaka's body of work undoubtedly pioneered our ability to culture spiny lobster phyllosoma with an emphasis on diet and water quality (Kittaka 1997). Here, we build on this progress having tested the success of culturing early- and middle-stage *P. argus* phyllosoma using a laminar flow, circular tank – the jellyfish plankton kreisel. To date, this work represents the first of its kind for such an application. Jellyfish kreisels have been at the forefront of successful gelatinous zooplankton culturing, and they offer an alternative approach to the mass production of spiny lobster phyllosoma (Sommer 1993). When combined with adequate food sources, good water quality, and consistent husbandry practices, these tanks show promising growth and survivorship for densities upwards of 5000 phyllosoma or ~28 animals $L^{-1}$.

### 4.1 Survival and density

It is not too surprising that two disparate density treatments did not affect the overall survival or growth of phyllosoma. Matsuda and Takenouchi (2005) cultured middle-stage Japanese spiny lobster (*Panulirus japonicus*) in 40 L elliptical tanks at densities of 40, 60, and 90 phyllosoma/tank (1.0, 1.5, and 2.25 animals $L^{-1}$, respectively), obtaining an 83% success rate through to late-stage development for all densities. In our study though, disease elicited an order of magnitude of difference in tanks with higher densities that warrants further study to determine the exact cause (e.g., intermolt duration, density) and the potential stressors involved. There did not seem to be a correlation of disease incidence with respect to molting events, however, certain feed sources, in particular jellyfish rations, were doubled for the higher densities and thus are considered a prime source of increased mortality, especially as phyllosomes increased their molt interval in later stages. Other studies have shown that complex marine microbial communities (i.e., biofilms) are regularly found in larval spiny lobster culture systems (e.g., *Panulirus ornatus*) and are capable of acting in concert with other factors such as high stocking densities and sub-optimal water quality, due to vibriosis (Bourne et al. 2004; Payne et al. 2006). However, flow and environmental conditions were no different for any of the kreisel tanks, and the incidence of outbreaks did not correlate to growth. Diagnostic results indicated that microbial communities were present in both tank densities throughout the study (especially evident in days 30–35) and seemed to be exacerbated by the increased phyllosoma density. The predominant group of bacteria identified, *Vibrio* sp., is common in marine waters and a causative agent of mortality when larvae are stressed. We suspect, a more vigilant approach to "clean" food sources (pre- and post-production feed methodology) is of paramount importance in lowering overall disease incidence and subsequent mortality.

### 4.2 Tank design

Tank designs vary greatly by shape and flow characteristics (see Matsuda and Takenouchi 2007; Table 2). Using a 40 L upwelling tank that uses mechanical rotation to create a gradual circular flow, Murakami (2004) was successfully able to raise phyllosoma from hatch to juvenile with a success rate of 28%. Illingworth et al. (1997) obtained an overall survival rate of 60% to Stage VIII for rock lobster (*Jasus edwardsii*) at a density of > 40 phyllosoma $L^{-1}$ using a multi-chambered upwelling tank. Still, others have been successful designing small (30–40 L) upwelling hemispherical tanks containing inputs of air and seawater from the bottom (Inoue 1981; Sekine et al. 2000). The hydrodynamics of a circular tank with a continuous, vertically-rotating current more than likely provides more natural conditions for phyllosoma to swim and avoid entanglement, as seen in this study. Other evidence suggests that circularly-rotating tanks serve as a better medium for late-stage phyllosoma (not tested exclusively in this study) that are preparing for metamorphosis to the postlarval (puerulus) stage (Matsuda pers. comm.). The final larval metamorphosis is a critical juncture in the life-phase of this species (McWilliams and Phillips 2007) and has been associated with high levels of mortality, especially when using sub-optimal tanks and flow rates (Matsuda and Takenouchi 2005). For example, phyllosoma late-stages ($n$ = 32) kept in a 100 L plankton kreisel with a rotating current of 1.5 L $min^{-1}$ successfully metamorphosed at a rate of 85%, compared with animals in non-rotating circular tanks (38%, $n$ = 32, Matsuda unpubl. data). Although it should be emphasized that this study was designed to take large numbers of phyllosoma into their middle stages, we did attempt to rear some phyllosoma ($n$ ~ 350) to the puerulus stage in one kreisel that was pooled from all tanks. However, due to logistical constraints, we terminated the study when animals reached Stage VIII or about three weeks from the puerulus stage. Despite this, we speculate that these tanks are capable of providing suitable conditions for growth through the entire larval phase when considering the ability to alter flow and circulation patterns from early- to late-stage development. The scaling down of these tanks to smaller ones, especially when considering realistic costs, remains a possibility (see Raskoff et al. 2003). Small numbers of early-stage phyllosoma have recently been reared through to the settlement stage (puerulus) in one other species of spiny lobster, (*Panulirus japonicus*) at a Japanese aquarium using a similar, but much smaller (40 L) kreisel tank design (T. Horita, unpub. data). Therefore, the potential to develop these tanks further for complete culture from hatch to settlement is optimistic.

### 4.3 Applications

In addition to providing good survival and growth at increased densities, circular kreisels such as these provide an exceptionally effective platform for conducting larval behavior experiments (e.g., ontogenetic vertical migration, feeding, aggregation, response to photoperiod). For example, using all clear tanks, live video recordings can be incorporated for studying feeding and swimming performances of the plankton phase of many larvae (e.g., jellyfish; Colin and Costello 2002) including phyllosoma. Also, the effect of tank color could be an important attribute in feeding behavior and even survivorship of these larvae as has been shown in mud crab larvae (Rabbani and Zeng 2005) and should be explored further. Obtaining these kinds of data would be a valuable contribution to our continued understanding for the culture success of this species and improve upon existing bio-physical modeling studies (Rudorff et al. 2009; Butler et al. 2011).

In considering phyllosoma culture tanks for land-based aquaculture, it is important to test, and potentially utilize, more than one design given the behavioral and physiological changes that ensue with changing growth and development throughout this 4 + month larval cycle (Butler et al. 2011). Episodic mass mortalities are substantiated by other studies that report that specific changes in tank design and flow characteristics may, in fact, increase the likelihood of successfully rearing phyllosoma past critical periods and provide the necessary physical attributes for the progression to key developmental stages (Matsuda and Takenouchi 2007). A relatively recent assessment of commercial aquaculture potential for *P. argus* comparing both land- and sea-based operations concluded that although sea-based culture methods would require less capital to establish, two other important aspects, (1) the development of a cost-effective, formulated diet; and (2) establishing densities for growout, would have to be considered (Jeffs and Davis 2003).

## 5 Conclusion

We suggest that for the mass production of land-based systems, a sharp focus should fall on building successful larval tank designs that will facilitate optimal growth and flow regimes that are conducive to food contact and the dispersal of phyllosoma and their unique body architecture at large densities through to settlement. A successful array of tank types suitable for the mass production of *P. argus* phyllosoma fills a large gap in linking the larval culture component of this species to the juvenile growout phase along with a nutritionally complete and cost effective feed.

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