Aquaculture xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864)

Miguel Costa Leal [a,b,*], Cristóvão Nunes [c,1], Sofia Engrola [c], Maria Teresa Dinis [c], Ricardo Calado [a]

[a] Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[b] Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA
[c] CCMAR—Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

ARTICLE INFO

Article history:
Received 4 August 2011
Received in revised form 27 March 2012
Accepted 29 March 2012
Available online xxxx

Keywords:
Anemone culture
Biomass
Temperature
Light

ABSTRACT

Sea anemones of genus *Aiptasia* are commonly used as biological models for biotechnological and molecular research. They are also employed to study the symbiotic interactions between cnidarians and zooxanthellae. In addition, *Aiptasia* is an important prey for the culture of the highly priced ornamental nudibranch *Aeolidiella stephanieae*. The purpose of this study was to determine the best culture conditions for establishing large monoclonal populations of this anemone. This study analyzed the effect of the following factors on *Aiptasia pallida* propagation and biomass increase throughout 60 days: initial anemone stocking density, light regimes, water temperature and different live diets. The best results were achieved at a higher water temperature (26 °C) and in darkness. *Artemia* nauplii were a better live prey than *Artemia* metanauplii to maximize biomass production, with lower initial anemone stocking densities maximizing propagation ratios. This research provides initial data that enables a large-scale production of monoclonal *A. pallida*, either to be used as a biological model, for the screening of new natural products or in the aquaculture of ornamental sea slugs.

1. Introduction

Sea anemones of the genus *Aiptasia*, commonly known as glass anemones, exhibit worldwide distribution, occurring from tropical to temperate marine ecosystems (Chen et al., 2008; Sunagawa et al., 2009). *Aiptasia* are known to possess a remarkable trophic plasticity. They are able to accomplish their energetic demands heterotrophically, by preying on zooplankton, and autotrophically, through the photosynthetates produced by their endosymbiotic microalgae — zooxanthellae (Bachar et al., 2007; Ruppert et al., 2003; Thorington and Hessinger, 1996). This symbiotic relationship can supply up to 95% of photosynthetically-fixed carbon to the host (Falkowski et al., 1984; Venn et al., 2008), which may allow some *Aiptasia* species to survive without any external food for long periods (Cook et al., 1988). Nonetheless, photosynthetates provided by the endosymbionts do not meet all the nutritional requirements of their cnidarian host, namely phosphorus and nitrogen, which play a crucial role in anemone growth and reproduction (Bachar et al., 2007).

Aiptasia hardiness in captivity, its fast growth rate and asexual reproduction, have allowed researchers to culture monoclonal individuals under controlled laboratorial conditions. Monoclonal individuals are produced when all specimens originate from a single parental organism, thus being genetically identical, which is a useful trait to control morphological and genetic plasticity (Weis et al., 2008). These features have contributed to make *Aiptasia* a successful biological model for research on reproduction (Chen et al., 2008; Clayton and Lasker, 1985), physiology (Davy and Cook, 2001), stress biology (Goulet et al., 2005), toxicology (Mercier et al., 1997), genetics (Kuo et al., 2004), natural products (Marino et al., 2004) and symbiotic relationships between cnidarians and zooxanthellae (Bachar et al., 2007; Davy and Cook, 2001; Mobley and Gleason, 2003; Muller-Parker, 1985). Furthermore, breeders of marine ornamental species culturing the aeollid nudibranch *Aeolidiella stephanieae* Valdéz 2005 are now targeting the production of large numbers of *Aiptasia*. Although erroneously, *A. stephanieae* is commonly known in the marine aquarium trade as *Berghia verrucicornis*. This small nudibranch feeds exclusively on these sea anemones and is universally employed to control these pests in reef aquariums (Carroll and Kempf, 1990; Kristof and Klussmann-Kolb, 2010). The efficiency of *A. stephanieae* to control *Aiptasia* is such that these nudibranchs commonly obtain high market prices (average retail price up to 20 € per specimen) in the marine aquarium trade. Curiously, the obstacle impairing the breeding of large numbers of *A. stephanieae*, thus the profitability of this activity, is the absence of a regular and abundant supply of their only prey — *Aiptasia* (Olivotto et al., 2011). Thus, it is of paramount importance to determine the best culture conditions for inexpensively establishing large monoclonal populations of glass anemones. The

* Corresponding author at: Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370350; fax: +351 234372587.
E-mail address: miguelcleal@gmail.com (M.C. Leal).
[1] Present address: Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal.

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2012.03.035

objective of the present study was to investigate the effect of different initial anemone stocking densities, light regimes, water temperatures and diets on the clonal propagation and biomass production of *Aiptasia pallida* (Agassiz in Verrill, 1864). The rationale for this approach was that higher stocking densities may enhance biomass production, but may slow down propagation rates. The supply of artificial light and heat was important to mimic natural sunlight and water temperature conditions, although it increases culture costs. On the other hand, the culture of anemones in dark and at low temperature may have notable drawbacks. While the culture of *A. pallida* in the absence of light prevents autotrophy, lower temperatures may reduce metabolic rates. Thus, growth and propagation rates may decrease. The effect of live feed leftover diets commonly drained from larviculture tanks was also investigated. *A. pallida* are commonly fed with pricey newly hatched *Artemia* nauplii, and the use of live feed leftovers, particularly *Artemia* metanauplii, reduces the cost of producing live food for *A. pallida*.

2. Material and methods

2.1. Husbandry and preliminary propagation of A. pallida

Monoclonal *A. pallida*, originating from a single specimen, were held in a 250-l culture tank (1 m × 0.5 m × 0.5 m) filled with 5 μm filtered and UV irradiated natural seawater. The tank was placed outdoors under natural sunlight and photoperiod at University of Algarve (Campus de Gambelas, Faro, Portugal). Ceramic tiles (20 mm × 20 mm) were positioned in the tank and lined up against the walls to increase available surface area for the growth of cultured anemones. The use of these ceramic tiles offered two additional advantages: 1) they allowed large numbers of *A. pallida* to be quickly collected from the culture tank; and 2) their smooth surfaces made it possible to easily release the anemones by gently scraping them at the base of their basal disk without inducing significant mechanical damage. Water temperature was kept at 26 °C. A 300 W electrical heater equipped with a thermostat was used to maintain water temperature. Salinity was kept stable at 35 ± 1 by daily adding freshwater to the culture tank (previously purified using a reverse osmosis unit) and thereby compensating any losses resulting from evaporation. Water circulation was provided by four airlifts placed at each corner of the culture tank. Nitrate levels varied between 10 and 20 mg l^{-1}, while ammonia and nitrite remained under detectable levels; pH values recorded were 8.0 ± 0.1. All parameters previously referred to were monitored using colorimetric tests (Tropic Marin®, Germany). Anemones were fed every three days with newly hatched *Artemia* nauplii (5 nauplii ml^{-1}; San Francisco Bay Brand Inc., USA) and allowed to freely propagate for 6 months prior to the start of the experiments. The tank bottom was siphoned once a week together with a 25% partial water change.

2.2. Size class determination

In order to determine the best non-destructive method to monitor anemones' biomass, forty anemones were randomly sampled from the culture tank, dried on absorbent paper and weighed (Sartorius scale; ±0.001 g). Samples were freeze-dried for 48 h and weighed again to determine the relationship between wet weight and dry weight. To examine the relationship between dry weight and pedal disk diameter (PDD) (Chomsky et al., 2004a), 70 *A. pallida* were randomly collected from the culture tank and the PDD measured. Measurements were performed using calipers (to the nearest 0.05 mm) after the anemone had retracted its tentacles and without removing the anemone from the ceramic plate. When necessary, the retraction of the anemone was stimulated with a plastic pipette in order to remove any excess water from inside the gastrovascular cavity. Each specimen was then scrapped off the ceramic plates and its dry weight was determined after freeze-drying for 48 h. Regression analysis supported the use of PDD for anemones' size measuring, and thus the following size classes were established for PDD: extra small (XS) (PDD<3 mm), small (S) (PDD between 3 and 6 mm), medium (M) (PDD between 6 and 9 mm) and large (L) (PDD>9 mm).

2.3. Determination of optimal prey concentration

Anemones from each size class (XS, S, M, L) were placed in separate 300 ml beakers with 5 μm filtered and UV irradiated natural seawater. The beakers were kept in a water bath at 26 °C and a 12 light (Lt):12 dark (D) photoperiod. The following number of anemones was used in each beaker for each size class: 6 specimens for sizes XS, S and M, and 3 specimens for size L. All experiments were made in triplicate, i.e., 3 different beakers were used for each size class. The anemones were fed with *Artemia* nauplii at the following concentrations: XS) 0.3, 0.6 and 1 nauplii ml^{-1}; S) 0.6, 1 and 1.3 nauplii ml^{-1}; M) 2, 3 and 4 nauplii ml^{-1}; and L) 5, 6.6 and 8.3 nauplii ml^{-1}. The range of prey concentration to be tested for each anemone size class had been previously determined in a preliminary experiment (unpublished data). After a period of 24 h, classification for prey ingestion was determined through visual observation, considering 4 levels of ingestion: partial ingestion, partial ingestion with regurgitation, total ingestion and total ingestion with regurgitation. Partial ingestion occurred when only a portion of all prey provided was ingested by the sea anemone. Partial ingestion with regurgitation was documented when only a portion of all prey provided was ingested together with regurgitated pack(s) of *Artemia* nauplii recorded in the bottom of the container. Total ingestion was defined as the ingestion of all prey provided. Total ingestion with regurgitation was defined when the ingestion of all prey provided was recorded together with the presence of regurgitated pack(s) of *Artemia* nauplii in the bottom of the container. Regurgitated pack(s) of Artemia nauplii varied in shape and size, although they were commonly 2–3 mm in diameter and displayed an orange coloration similar to the prey provided to *A. pallida*.

2.4. Effect of initial stocking density, light regime, water temperature and diet on clonal propagation and biomass production

Triplicate trials (each performed in 3 l plastic tanks (0.1 m × 0.1 m × 0.3 m)) were performed for each possible combination of the following factors: initial anemone stocking density (90 vs. 180 anemones m^{-2}), light regime (12 h Lt:12 h D vs. 24 h D), water temperature (22 vs. 26 °C, regulated through a water heating/cooling device) and diet (*Artemia* nauplii resulting from newly hatched nauplii (hereafter referred to as nauplii) vs. *Artemia* metanauplii resulting from nauplii enriched with *Tetraselmis chui* and *Isochrysis galbana* during 24 h (hereafter referred to as metanauplii)). The experiment lasted 60 days and was performed with XS (PDD<3 mm), S (PDD between 3 and 6 mm) and M (PDD between 6 and 9 mm) anemones. In order to meet the initial anemone stocking densities selected for the present study, the following combination of anemones was selected: 3 XS, 3 S and 3 M anemones (for the 90 anemones m^{-2} treatment) and 6 XS, 6 S and 6 M anemones (for the 180 anemones m^{-2} treatment). We only considered the bottom area (0.1 m^2) of plastic tanks in order to achieve the initial stocking densities. Stocked anemones were fed every 3 days, as preliminary tests verified that this was the average time required for complete food digestion. The number of prey supplied to each tank was calculated in accordance with the results of the experiment described above to determine optimal prey concentration. The following formula was used to calculate the number of prey to provide based on the number of anemones of each size class per tank: (number of XS anemones × 100 prey) + (number of S anemones × 300 prey) + (number of M anemones × 900 prey) + (number of L anemones × 1500). The number of prey was adjusted after counting the number of anemones in each tank. The adjustment was derived from the previous formula, along with results from anemone counts. Anemones were counted and

Please cite this article as: Leal, M.C., et al., Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864), Aquaculture (2012), doi:10.1016/j.aquaculture.2012.03.035

categorized in size classes 15 (t_{15}), 30 (t_{30}), 45 (t_{45}) and 60 (t_{60}) days after the beginning of the experiment (t_0). Every week the bottom of the tanks was siphoned with a pipette to avoid aspiration of small anemones and 50% of the water was replaced. Water quality parameters were monitored as described above for the culture tank. Anemone's clonal propagation was determined by the ratio between number of anemones at t_x/ number of anemones at t_0. Average anemone biomass (wet weight) per replicate was determined using the regression equations referred above for the classification of anemone's size classes (see Fig. 1). In order to facilitate the estimation of anemones' biomass in each culture tank, the number of specimens in each size class was counted and multiplied by the average wet weight of each size class. The average wet weights were also calculated, using the results obtained from the size class determination essays (XS) 7.37 mg; S) 52.77 mg; M), 213.38 mg; and L 489.21 mg).

2.5. Statistical analysis

Simple regression analysis was used to determine the relation between wet weight and dry weight, as well as between dry weight and PDD. Anemone's biomass production and clonal propagation were analyzed for the following factors: initial anemone stocking density (2 levels), light regimes (2 levels), water temperature (2 levels), diet (2 levels) and time (4 levels). As measurements were taken from the same tanks at t_{15}, t_{30}, t_{45} and t_{60}, repeated measures 4-way ANOVA (rm-ANOVA) was used. Assumptions of rm-ANOVA were tested and whenever necessary data was transformed (square root) to meet the assumptions (Zar, 1998). Tukey HSD test was used when rm-ANOVAs revealed significant differences ($p<0.05$). All statistical analyses were performed using STATISTICA 8.0 software (StatSoft Inc.).

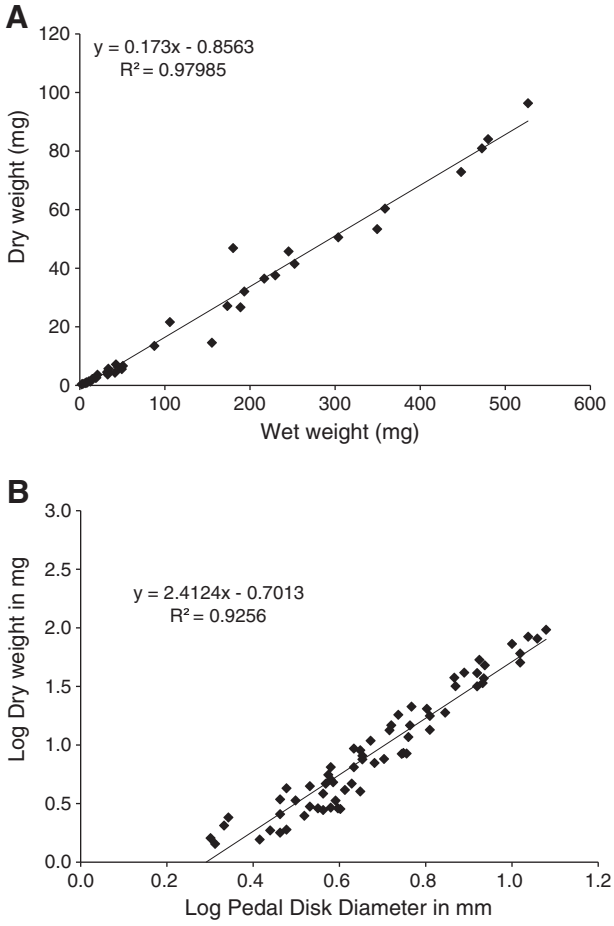

Fig. 1. Relationship between dry weight and wet weight (A) and the logarithm of dry weight and the logarithm of the pedal disk diameter (B) of the sea anemone *A. pallida*. Linear (A) and logarithmic (B) regression lines are presented.

3. Results

3.1. Size class determination

A. pallida possessed an average water content of 84.9%. A positive and significant linear association was observed between anemone wet and dry weight (Fig. 1A; $F=1847.435$, $p<0.0001$). A positive and significant logarithmic association was also observed between dry weight and pedal disk diameter (PDD) (Fig. 1B; $F=433.925$, $p<0.0001$). These results supported the use of both wet weight and PDD for all subsequent analysis in the present study concerning *A. pallida* growth.

3.2. Determination of optimal prey concentration

The prey ingestion observed for the different concentrations is summarized in Table 1. For XS anemones, the only prey concentration that resulted in feeding behaviors different from partial ingestion with regurgitation was 0.3 nauplii ml^{-1}. For S specimens, regurgitation or total ingestion was only avoided when a prey concentration of 1 nauplii ml^{-1} was provided. For M anemones, 100% of total ingestion and 100% of partial ingestion with regurgitation were observed for concentrations of 2 and 4 nauplii ml^{-1}, respectively. For L anemones, regurgitation was only avoided with a prey concentration of 5 nauplii ml^{-1}.

3.3. Effect of initial stocking density, light regime, water temperature and diet on clonal propagation and biomass production

Clonal propagation ratios varied with tested factors throughout the experiment. At t_{60}, significantly higher propagation ratio ($p<0.01$; 10.1 ± 1.0, average $\pm$ SD) was observed in tanks initially stocked with lower anemone density (90 anemones m^{-2}) compared to tanks with higher anemone density (7.9 ± 1.0; 180 anemones m^{-2}). Significantly higher propagation ratios ($p<0.01$) were also observed with higher water temperature (ratio at 22 °C $=7.7\pm0.7$; ratio at 26 °C $=10.3\pm1.3$), as well as in anemones cultured in total darkness

Table 1
Observations of feeding behaviors displayed by different size classes of *A. pallida* when provided different concentrations of newly hatched *Artemia* nauplii.

A. pallida size class	Prey concentrations (nauplii ml^{-1})	Feeding behavior (%)
Extra small	0.3	PIR (33.3)
		TI (33.3)
		TIR (33.3)
	0.6	PIR (100)
	1	PIR (100)
Small	0.6	TI (100)
	1	PI (100)
	1.3	PI (33.3)
		PIR (66.6)
Medium	2	TI (100)
	3	TI (66.6)
		TIR (33.3)
	4	PIR (100)
Large	5	PI (33.3)
		TI (66.6)
	6.6	PI (33.3)
		PIR (33.3)
		TIR (33.3)
	8.3	PIR (66.6)
		TI (33.3)

Feeding behaviors: partial ingestion (PI), partial ingestion with regurgitation (PIR), total ingestion (TI), total ingestion with regurgitation (TIR).

Please cite this article as: Leal, M.C., et al., Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864), Aquaculture (2012), doi:10.1016/j.aquaculture.2012.03.035

($p<0.01$; ratio in Lt = 6.9 ± 0.9; ratio in D = 11.1 ± 1.0) and with nauplii as prey ($p<0.01$; nauplii = 10.5 ± 1.2; metanauplii = 7.5 ± 0.8). When accounting for the interaction of all factors, significant interactions were observed between light regime, temperature and prey. Anemones reared in the dark, at 26 °C and fed with nauplii recorded propagation ratios significantly higher than other anemones (ratio of 18.9 at t_{60} for 90 anemones m^{-2} and 13.7 at t_{60} for 180 anemones m^{-2}; $p<0.01$ for both initial stocking densities). In contrast, the lowest propagation ratios at t_{60} were recorded for anemones exposed to light, reared at 22 °C and fed with metanauplii (4.8 at for 90 anemones m^{-2} and 5.3 at for 180 anemones m^{-2}). No significant differences were observed when considering 3 or more tested factors ($p>0.17$), apart from the interaction between water temperature, light and diet ($p<0.01$).

For both light regimes and initial stocking density treatments, the highest biomass recorded at t_{60} was achieved for anemones cultured at 26 °C and fed with nauplii (Fig. 2). The only exception was observed for the treatments in total darkness, higher initial anemone stocking density, when provided with nauplii and water temperature of 22 or 26 °C (Fig. 2D). However, no significant differences were observed between these two treatments (Tukey HSD, $p = 0.92$). Regarding overall biomass production, water temperature induced significant changes ($df = 1$, $F = 13.38$, $p<0.01$), in contrast to light regime ($df = 1$, $F = 0.02$, $p = 0.88$). Even so, significant interactions between light, diet, water temperature and time were observed for biomass production ($df = 3$, $F = 4.52$, $p<0.01$). At t_{60}, the only significant factors were initial anemone stocking density ($df = 1$, $F = 191.3$, $p<0.01$) and prey ($df = 1$, $F = 68.8$, $p<0.01$). Lower biomass production was observed in tanks with lower initial anemone stocking density and also observed when metanauplii were provided. The highest biomass production for the higher initial anemone stocking density was recorded for the treatment exposed to dark conditions (3.61 mg wet weight, at 22 °C and fed nauplii; Fig. 2D). In contrast, for the lower initial anemone stocking density the highest biomass production was observed for the tank exposed to a 12 h Lt:12 h D (2.7 mg wet weight; 26 °C and fed nauplii; Fig. 2A).

The average proportion of different anemone size classes per treatment is presented in Fig. 3. A noteworthy higher abundance of XS anemones was observed in all tanks (66.7 to 94.7%), while L anemones were the scarcest. Medium and L anemones were almost twice as abundant in light as in dark treatments (average cumulative percentage: Lt — 2.3%, D — 1.3%).

4. Discussion

Anemones are soft-bodied invertebrates that display the ability to store significant amounts of water in their body cavity. Therefore, the precise measurement of anemones using non-destructive techniques is a challenging task. Different morphological criteria have been used to estimate anemone size. For instance, Clayton and Lasker (1985) used the oral disk diameter for measuring *A. pallida*, while Hand and Uhlinger (1992) used the column length to measure *Nematostella vectensis* Stephenson, 1935. In the case of *Actinia equina* (Linnaeus, 1758), Chomsky et al. (2004b) used the pedal disk diameter (PDD). Results observed for *A. pallida* are similar to those Chomsky et al. (2004b) observed for *A. equina*. Our results indicate that PDD provides a good morphological feature for non-destructive measurement of *A. pallida* biomass. In addition, due to anemone retraction and/or water expulsion from the coelenteron, the measurement of the PDD is simpler to accomplish and less prone to variation than other methods commonly used, such as, wet weight, oral disk diameter and column length. The authors recommend that future studies addressing other species of anemones might also evaluate the suitability of using PDD to determine anemone biomass.

Previous studies that maintained live stocks of anemone and/or stimulated anemone growth, either fed the anemones to repletion or omitted any information concerning the amount of prey provided to stocked specimens (Chomsky et al., 2004a, 2004b; Cook et al., 1988). Feeding anemones to repletion requires the provision of extra food, which wastes unnecessary food resources and accelerates water quality deterioration. When anemones ingest excess food they often regurgitate, which also contributes to the deterioration of water quality. Therefore, the amount of food provided should be adjusted to the number and size of anemones present in culture tanks, thus

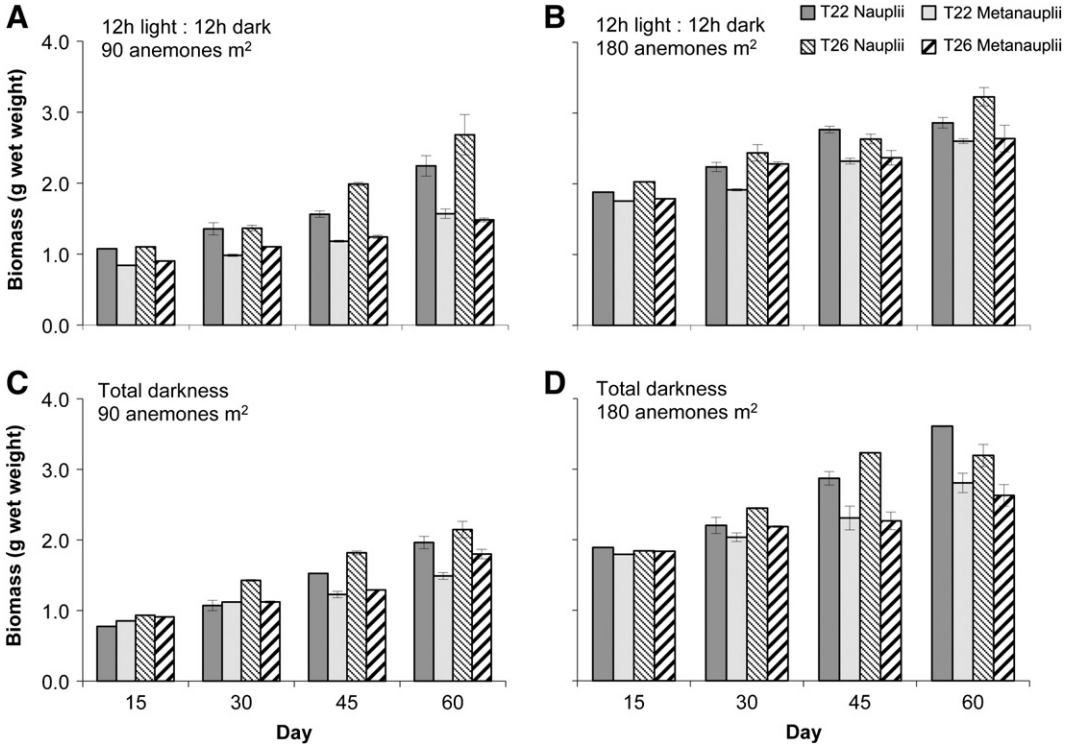

Fig. 2. Variation of biomass (g of wet weight) of *A. pallida* per tank throughout the experiment when reared with different conditions (light—A, B; dark—C, D; 90 anemones m^{-2}—A, C; 180 anemones m^{-2}—B, D). Error bars designate standard error.

Please cite this article as: Leal, M.C., et al., Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864), Aquaculture (2012), doi:10.1016/j.aquaculture.2012.03.035

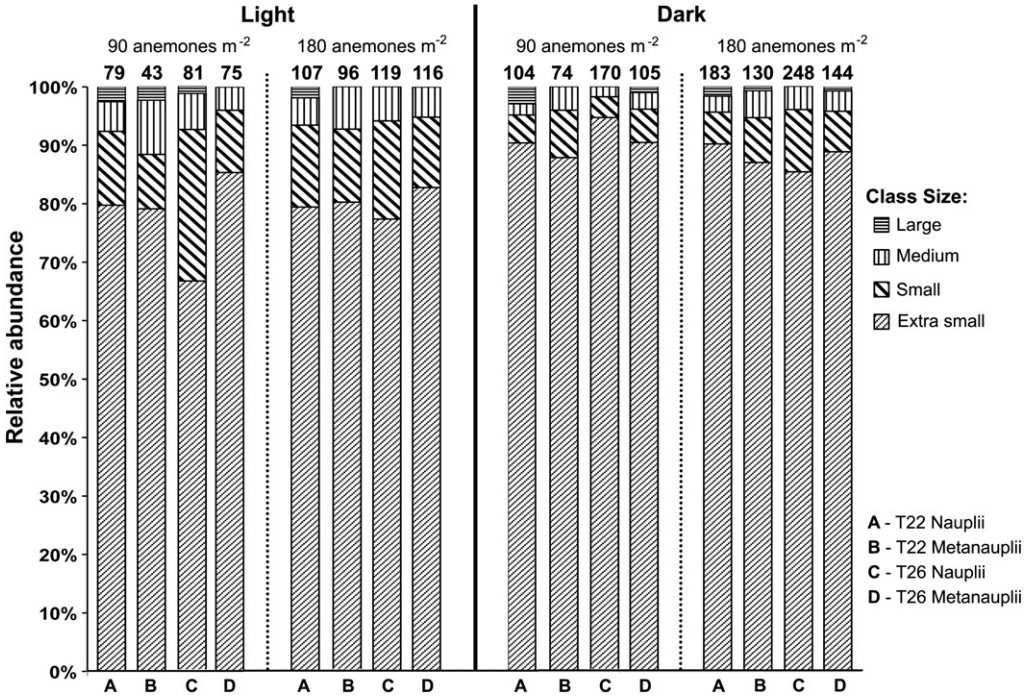

Fig. 3. Average proportion of *A. pallida* of different size classes after 60 days (t_{60}) for different light, initial stocking density, water temperature and diet conditions. Total number of anemones is also presented above the bar of each treatment (A—22 °C and nauplii; B—22 °C and metanauplii; C—26 °C and nauplii; D—26 °C and metanauplii).

avoiding food wastage, regurgitation and deterioration of water quality. A scenario yielding ideal results should be between partial and total ingestion without regurgitation. Total ingestion may suggest that anemones are not fed to repletion while partial ingestion indicates food wastage. In the present study, the most suitable prey concentrations per anemone size class that avoided partial ingestion with regurgitation and/or total ingestion scenarios were 0.3, 1, 3 and 5 nauplii l^{-1} for XS, S, M and L sized anemones, respectively.

The optimization of prey concentration was critical to assure that during the experimental trials enough food was being supplied to *A. pallida*. Higher propagation ratio and biomass production of cultured *A. pallida* were observed when higher water temperature was used (26 °C) together with total darkness conditions. This was not unexpected, as *A. pallida* commonly occurs in tropical regions, to which this species is well adapted. Lower water temperature commonly favors the propagation of temperate species, such as *A. equina* (Chomsky et al., 2004a). The same rationale may be used to explain why the highest water temperature (26 °C) also favored the production of *A. pallida* biomass. These results suggest that in order to maximize biomass production one should adjust water temperature to that of the latitudinal distribution of the target anemone species and avoid thermal stress. Higher propagation ratios were also obtained in tanks with lower initial anemone stocking densities, suggesting that space availability is also an important factor to consider when targeting the mass culture of anemones. Our results also suggest that initial anemone density can be a critical factor for optimization of biomass production, since it may negatively affect it. In some particular culture conditions a decrease in biomass production was observed. This was probably associated with elevated rates of pedal laceration that increased the numbers of small anemones (see Fig. 3).

A. pallida commonly have zooxanthellae and thus can benefit from light to survive without feeding heterotrophically (Bachar et al., 2007; Venn et al., 2008). On the other hand, when this species is kept under total darkness it eventually becomes azooxanthellate and loses any energetic income provided by photosynthetic endosymbionts. For this reason, one should expect light to benefit the production of *A. pallida*, both in numbers and biomass. However, previous studies reported no differences on propagation ratios between *Anthopleura elegantissima* (Brandt, 1835) exposed to light or kept in total darkness (Sebens, 1980), nor between zooxanthellate and azooxanthellate *A. pallida* (Clayton and Lasker, 1985). As reported above, in our study *A. pallida* reared in total darkness generally presented higher propagation ratios and biomass than those reared in light. The higher propagation ratios recorded for anemones in the absence of light may be associated with an enhancement of pedal laceration promoted by anemone displacement within the rearing tank while searching for suitable light conditions (Geller et al., 2005). This behavior enhances propagation ratios, as peduncle laceration is likely to occur whenever *A. pallida* move.

Both nauplii and metanauplii were readily ingested by *A. pallida*, although propagation ratios and biomass increase was favored by nauplii. These results may be explained by the smaller body size displayed by nauplii, which can make them more vulnerable to predation by *A. pallida*, or by a higher nutritional quality of nauplii than metanauplii. Nevertheless, the present study opens good perspectives for the potential use of live feed leftovers from marine hatcheries.

Large scale production of monoclonal *A. pallida*, as well as other *Aiptasia* species, cultured under optimal and replicable conditions may allow researchers to have a regular supply of high quality and genetically identical specimens to be used as biological models in several research fields, namely for studying cnidarian–zooxanthellae symbiotic relationships (as suggested by Weis et al. (2008)). Additionally, researchers screening for new marine natural products from anemones can eliminate two of the most serious problems commonly faced in this research field: 1) the loss of the source — as anemones will continuously be available under culture; and 2) reproducibility — as monoclonal specimens produced under exactly the same conditions can be used for future trials along the pipeline for the discovery of new drugs (Leal et al., 2012; Li and Vederas, 2009; Rocha et al., 2011). The present study provides information that will assist in the development of large scale, monoclonal production of *A. pallida*; however, further research is necessary to understand if the production of these organisms in the absence of light (as suggested by this study) will influence their physiology, gene expression or metabolic pathways once they become infected, or re-infected, by zooxanthellae. Furthermore, the monoclonal production of *A. pallida* using the effluents of industrial hatcheries

Please cite this article as: Leal, M.C., et al., Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864), Aquaculture (2012), doi:10.1016/j.aquaculture.2012.03.035

should also be tested in future studies. Besides the advantage of re-using live feed leftovers, *A. pallida* may also remove organic nutrients from the effluent. Symbiotic anemones harbor photosynthetic endosymbionts in their tissues, which need nutrients to produce photosynthetates. Eventually, by up taking these nutrients, anemones' endosymbionts will decrease their load in effluents and contribute to improve water quality. This last aspect of effluent bio-remediation will only be possible if light is either naturally available or supplied to the anemones, in order to sustain their photosynthetic endosymbionts.

In conclusion, to optimize the monoclonal production of *A. pallida*, the present results suggest the use of the following conditions: 26 °C, total darkness and *Artemia* nauplii. Nevertheless, the duration of this study was only 60 days, and the optimal conditions for long-term culture of *A. pallida* remain to be determined. The initial anemone stocking density to be used should be adjusted to the purpose of the monoclonal production: 1) higher stocking densities to enhance anemone asexual reproduction and thus result in higher number of small anemones (of paramount importance for the culture of newly metamorphosed ornamental sea slugs *A. stephanieae*); or 2) lower stocking densities to promote biomass production.

Acknowledgments

Miguel C. Leal and Sofia Engrola acknowledge financial support by Fundação para a Ciência e Tecnologia, Portugal, through grants SFRH/BD/63783/2009 and SFRH/BPD/49051/2008, respectively. We also thank Nancy Tenenbaum for revising the manuscript and three anonymous reviewers for their comments and suggestions to improve the manuscript.

References

Bachar, A., Achituv, Y., Pasternak, Z., Dubinsky, Z., 2007. Autotrophy versus heterotrophy: the origin of carbon determines its fate in a symbiotic sea anemone. Journal of Experimental Marine Biology and Ecology 349, 295–298.

Carroll, D., Kempf, S., 1990. Laboratory culture of the aeolid nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): some aspects of its development and life history. Biology Bulletin 179, 243–253.

Chen, C., Soong, K., Chen, C., 2008. The smallest oocytes among broadcast-spawning actiniarians and a unique lunar reproductive cycle in a unisexual population of the sea anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria). Zoological Studies 47, 37–45.

Chomsky, O., Kamenir, Y., Hyams, M., Chadwick-Furman, N., 2004a. Effects of temperature on growth rate and body size in the Mediterranean Sea anemone *Actinia equina*. Journal of Experimental Marine Biology and Ecology 313, 63–73.

Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., Chadwick-Furman, N., 2004b. Effects of feeding regime on growth rate in the Mediterranean Sea anemone *Actinia equina* (Linnaeus). Journal of Experimental Marine Biology and Ecology 299, 217–229.

Clayton Jr., W., Lasker, H., 1985. Individual and population growth in the asexually reproducing anemone *Aiptasia pallida* Verrill. Journal of Experimental Marine Biology and Ecology 90, 249–258.

Cook, C., D'Elia, C., Muller-Parker, G., 1988. Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida*. Marine Biology 98, 253–262.

Davy, S., Cook, C., 2001. The relationship between nutritional status and carbon flux in the zooxanthellate sea anemone *Aiptasia pallida*. Marine Biology 139, 999–1005.

Falkowski, P., Dubinsky, Z., Muscatine, L., Porter, J., 1984. Light and the bioenergetics of a symbiotic coral. BioScience 34, 705–709.

Geller, J., Fitzgerald, L., King, C., 2005. Fission in sea anemones: integrative studies of life cycle evolution. Integrative and Comparative Biology 45, 615–622.

Goulet, T., Cook, C., Goulet, D., 2005. Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the *Aiptasia pallida*/*Symbiodinium* symbiosis. Limnology and Oceanography 50, 1490–1498.

Hand, C., Uhlinger, K., 1992. The culture, sexual and asexual reproduction, and growth of the sea anemone *Nematostella vectensis*. Biology Bulletin 182, 169–176.

Kristof, A., Klussmann-Kolb, A., 2010. Neuromuscular development of *Aeolidiella stephanieae* Valdez, 2005 (Mollusca, Gastropoda, Nudibranchia). Frontiers in Zoology 7, 5.

Kuo, J., Chen, M.-C., Lin, C.-H., Fang, L.S., 2004. Comparative gene expression in the symbiotic and aposymbiotic *Aiptasia pulchella* by expressed sequence tag analysis. Biochemical and Biophysical Research Communications 318, 176–186.

Leal, M.C., Puga, J., Serôdio, J., Gomes, N.C.M., Calado, R., 2012. Trends in the discovery of new marine natural products from invertebrates over the last two decades — where and what are we bioprospecting? PLoS One 7, e30580.

Li, J.W.-H., Vederas, J.C., 2009. Drug discovery and natural products: end of an era or an endless frontier? Science 325, 161–165.

Marino, A., Valveri, V., Muia, C., Crupi, R., Rizzo, G., Musci, G., Laspada, G., 2004. Cytotoxicity of the nematocyst venom from the sea anemone *Aiptasia mutabilis*. Comparative Biochemistry and Physiology. C 139, 295–301.

Mercier, A., Pelletier, É., Hamel, J.-F., 1997. Effects of butyltins on the symbiotic sea anemone *Aiptasia pallida* (Verrill). Journal of Experimental Marine Biology and Ecology 215, 289–304.

Mobley, K., Gleason, D., 2003. The effect of light and heterotrophy on carotenoid concentrations in the Caribbean anemone *Aiptasia pallida* (Verrill). Marine Biology 143, 629–637.

Muller-Parker, G., 1985. Effect of feeding regime and irradiance on the photophysiology of the symbiotic sea anemone *Aiptasia pulchella*. Marine Biology 90, 65–74.

Olivotto, I., Planas, M., Simões, N., Holt, G., Avella, M., Calado, R., 2011. Advances in breeding and rearing marine ornamentals. Journal of the World Aquaculture Society 42, 135–166.

Rocha, J., Peixe, L., Gomes, N.C.M., Calado, R., 2011. Cnidarians as a source of new marine bioactive compounds—an overview of the last decade and future steps for bioprospecting. Marine Drugs 9, 1860–1886.

Ruppert, E., Fox, R., Barnes, R., 2003. Invertebrate Zoology: A Functional Approach, 7th edition ed. Brookes Cole, Belmont, USA.

Sebens, K., 1980. The regulation of asexual reproduction and indeterminate body size in the sea anemone *Anthopleura elegantissima* (Brandt). Biology Bulletin 158, 370–382.

Sunagawa, S., Wilson, E.C., Thaler, M., Smith, M.L., Caruso, C., Pringle, J.R., Weis, V.M., Medina, M., Schwarz, J.A., 2009. Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. BMC Genomics 10, 258.

Thorington, G., Hessinger, D., 1996. Efferent mechanisms of discharging cnidae: I. Measurements of intrinsic adherence of cnidae discharged from tentacles of the sea anemone, *Aiptasia pallida*. Biology Bulletin 190, 125–138.

Venn, A., Loram, J., Douglas, A., 2008. Photosynthetic symbioses in animals. Journal of Experimental Botany 59, 1069–1080.

Weis, V., Davy, S., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., Pringle, J., 2008. Cell biology in model systems as the key to understanding corals. Trends in Ecology & Evolution 23, 369–376.

Zar, J., 1998. Biostatistical Analysis, 4th ed. Prentice Hall International, New Jersey.

Please cite this article as: Leal, M.C., et al., Optimization of monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864), Aquaculture (2012), doi:10.1016/j.aquaculture.2012.03.035