Zoological Studies **60**:38 (2021)
doi:10.6620/ZS.2021.60-38

Zoological Studies

Open Access

Seasonal Growth of the Purple Sea Urchin *Heliocidaris crassispina* Revealed by Sequential Fluorochrome Tagging

Juan Diego Urriago Suarez[1,2,3], Jane C.Y. Wong[2], Gilbert Lui[4], Clement P. Dumont[5], Jian-Wen Qiu[2,3], and Monthon Ganmanee[1,*]

[1]*Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand. *Correspondence: E-mail: monthon.ga@kmitl.ac.th (Ganmanee). Tel: +6623298140. E-mail: urriago@gmail.com (Suarez)*
[2]*Department of Biology, Hong Kong Baptist University, Hong Kong, China. E-mail: chingyanw11@gmail.com (Wong)*
[3]*Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), China. E-mail: qiujw@hkbu.edu.hk (Qiu)*
[4]*Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China. E-mail: csglui@gmail.com (Lui)*
[5]*School of Biological Sciences, The University of Hong Kong, Hong Kong, China. E-mail: clement@asc.run (Dumont)*

Received 8 January 2020 / Accepted 6 May 2021 / Published 13 July 2021
Communicated by Yoko Nozawa

Many studies have applied fluorochrome tagging to examine the growth of animals with calcified skeletons, but most of them have used only a single tag to determine the annual growth rate. We used sequential fluorochrome tagging to study the seasonal growth of the purple sea urchin *Heliocidaris crassispina* in Hong Kong waters from February 2012 to February 2013. Sea urchins ranging from 18.9 to 42.7 mm in test diameter had a yearly growth from 0.6 to 13.0 mm. During that year, the sea urchins grew from 0.6 to 5.0 mm in test diameter during the first six months, and from 0.4 to 10.2 mm in test diameter in the second six months. The seasonal differences in growth were confirmed using the von Bertalanffy model. The growth was clear for young sea urchins, especially for individuals less than 5 years old, but was not evident for sea urchins older than 7 years. The seasonal differences in growth were probably related to the reproductive cycle and the seasonal differences in environmental conditions. Our empirical results provide the first evidence of seasonal changes in growth for *H. crassispina*, demonstrating the usefulness of sequential fluorochrome tagging in studying the growth of sea urchins in the field. We also identify the problem of low recovery of tagged individuals and provide recommendations to improve the tagging procedure.

Key words: Calcein, Calcein blue, Fluorochrome, Seasonal growth model, Sea urchin, Tagging.

BACKGROUND

Due to a high market demand, sea urchins are widely harvested and many sea urchin stocks around the world have declined (Stefánsson et al. 2017). *Heliocidaris crassispina* is among the 20 sea urchins species with the highest market demand worldwide; the most exploited species are *Mesocentrotus franciscanus* (previously as *Strongylocentrotus franciscanus*), *Strongylocentrotus pulcheriius*, and *S. intermedius* (Stefánsson et al. 2017). Growth models provide information on population dynamics, and their parameters reflect environmental conditions and fishing pressure. These models can predict population growth rates and permit better management of biological resources based on demographic parameters (Ebert 2013).

Different techniques have been used to estimate the growth of sea urchins (review by Ebert 2020): analysis of natural growth lines and size-frequency

Citation: Suarez JDU, Wong JCY, Lui G, Dumont CP, Qiu JW, Ganmanee M. 2021. Seasonal growth of the purple sea urchin *Heliocidaris crassispina* revealed by sequential fluorochrome tagging. Zool Stud **60**:38. doi:10.6620/ZS.2021.60-38.

distributions, use of internal tags (PIT tags) and invasive tags (tethered through the test), and tagging skeletal parts using fluorochromes. In field studies, internal tagging using fluorochromes (*e.g.*, tetracycline, calcein, alizarin complexone) has been a widely adopted method for studying the growth of sea urchins over the past 60 years, although most such studies have used only a single fluorochrome (Campbell et al. 2001; Kobayashi and Taki 1969; Russell and Urbaniak 2004; Stuart and Smith 1992). Ellers and Johnson (2009) was the first study to use polyfluorochrome tagging on invertebrates (sea urchins), although the trials were conducted in the laboratory only (Ellers and Johnson 2009; Johnson et al. 2013). Several studies showed that the use of tetracycline or calcein had little or no negative effect (Ebert 1988; Kalvass et al. 1998) or only a transient effect on sea urchin survival or growth (Gage 1991; Russell and Urbaniak 2004). These fluorochromes produce a growth mark on various calcified elements (*i.e.*, jaws and test ossicles) that can be seen using a fluorescent microscope (Ellers and Johnson 2009).

Heliocidaris crassispina is a western Pacific species with a wide latitudinal distribution (38°N to 19°N) and is found in intertidal and subtidal rocky shores (usually up to 15 m deep) with strong wave action. This edible species is the only sea urchin commercially harvested in Hong Kong (Agatsuma 2007). Its overexploitation Chiu (1987), however, highlights the need for a better knowledge of the growth of *H. crassispina* for a better management of this species. Information about seasonal growth rates of sea urchins can help us understand their population dynamics and responses to harvesting activities (Ebert 1999). There have been studies of the population dynamics of *H. crassispina* in Hong Kong (Chiu 1987) and Japan (Agatsuma 2013), as well as modelling of its growth in these two places by counting and measuring growth rings on genital plates (Chiu 1990; Yatsuya and Nakahara 2004). Lau et al. (2011) estimated the annual growth of *H. crassispina* (as *Anthocidaris crassispina*) in Hong Kong waters by making one release of sea urchins tagged with the fluorochrome calcein and collecting them one year later. They found that the Jolicoeur model provided a better fit for the growth of *H. crassispina* at Nine Pins than did the von Bertalanffy model. The seasonal growth model used in our study was restricted to the von Bertalanffy model because more complex models such as the Jolicoeur model with seasonal growth have not been developed.

Using *H. crassispina* as the study animal, we explored the use of sequential tagging with two fluorochromes to examine the growth in the field for the first time in sea urchins. Our aim was to determine whether this technique can be used to study sea urchin skeletal growth seasonality in the field.

MATERIALS AND METHODS

Study site

Our study was conducted in the Hong Kong Special Administrative Region (HKSAR), situated along the southeastern coast of the People's Republic of China (lat. 22°09–22°37N) within the Tropic of Cancer (Fig. 1). Hong Kong has a tropical climate with a dry season (*i.e.*, winter) from November to April and a wet season (*i.e.*, summer) from May to October (Observatory 2013). Our study was conducted from February 2012 to February 2013 at Nine Pins (22°26'70.19"N, 114°34'60.89"E) in Mirs Bay, where the water is characterized as oceanic with stable salinities of 32–33 PSU and water temperatures between 17°C in winter and 28°C in summer (EPD 2012). The study site was wave-exposed on a moderate sloped bottom, supporting a rocky barrens community dominated by *Heliocidaris crassispina* at 3 to 6 m water depth.

Polyfluorochrome sequential marking

We used calcein and calcein blue fluorescent tags in this study. As the two fluorochromes have different excitation peaks (495 nm for calcein and 360 nm for calcein blue) and emission peak wavelengths (515 nm for calcein and 445 nm for calcein blue), different filters were used to observe the fluorescent marks (Pautke et al. 2005): FITC (Fluorescein - Isothiocyanate with an excitation peak of 490 nm and an emission peak of 520 nm) for calcein (green color), and DAPI (4, 6-Diamidino-2-Phenylindole with an excitation peak of 372 nm and an emission peak of 456 nm) for calcein blue (blue color).

To evaluate the seasonal growth of *H. crassispina*, we collected 827 individuals on 13 February 2012 (Season 1) from an area of ~47 m^2 and tagged them with calcein. On 30 August 2012 (Season 2), we collected 803 *H. crassispina* from the same location and tagged them with calcein blue. Based on the results of Lau et al. (2011), we estimated that these numbers of sea urchins would provide enough tagged individuals to construct reliable growth models. To evaluate growth during the first six months we collected and dissected an additional 50 sea urchins. Finally, on 26 February 2013 (~1 year after tagging), we collected 412 sea urchins from the same location (individuals that may have had both fluorochromes marks). All sea urchins collected for tagging were transported to the Swire Institute of Marine Science within 90 min and then temporarily

maintained in outdoor aerated tanks (60 cm × 70 cm × 90 cm) supplied with flow-through seawater (~200 sea urchins per tank) and protected from direct sunlight by an opaque roof (Fig. S1).

Before tagging, the sea urchins were placed in the outdoor tanks with food (the macroalgae *Ulva lactuca* and *Sargassum hemiphyllum*, and commercial lettuce) for two days. The test diameter of each sea urchin was measured to the nearest 0.1 mm using digital calipers. Individuals > 20 mm in test diameter were tagged by injecting 2.0 ml of the tagging solution (500 ppm of fluorochrome in natural filtered seawater buffered to pH 8 with NaOH) into the coelom using a syringe (Ellers and Johnson 2009; Johnson et al. 2013; Lamare and Mladenov 2000) (Fig. S1). Smaller individuals (≤ 20 mm test diameter) were placed in a fluorochrome bath for 24 h (n = 86) with the same concentration used for the injections (n = 1544) (Fig. S2). One day after tagging, the sea urchins were returned to the field collection site. The sea urchins behaved normally and there was no mortality before they were released back into the field. To facilitate the recapture, the area of urchin release (47 m^2) was marked by four steel rods hammered into rock crevices. A previous study indicated that this species of sea urchin has very little mobility (Freeman 2003).

At the end of the experiment, the sea urchins were collected, measured (using a digital caliper ± 0.1 mm), their Aristotle's lanterns extracted, and immersed in bleach (sodium hypochlorite with 5% active chlorine) for 24 h to remove the soft tissues. The demi-pyramids (jaws) were then rinsed with freshwater and air-dried for

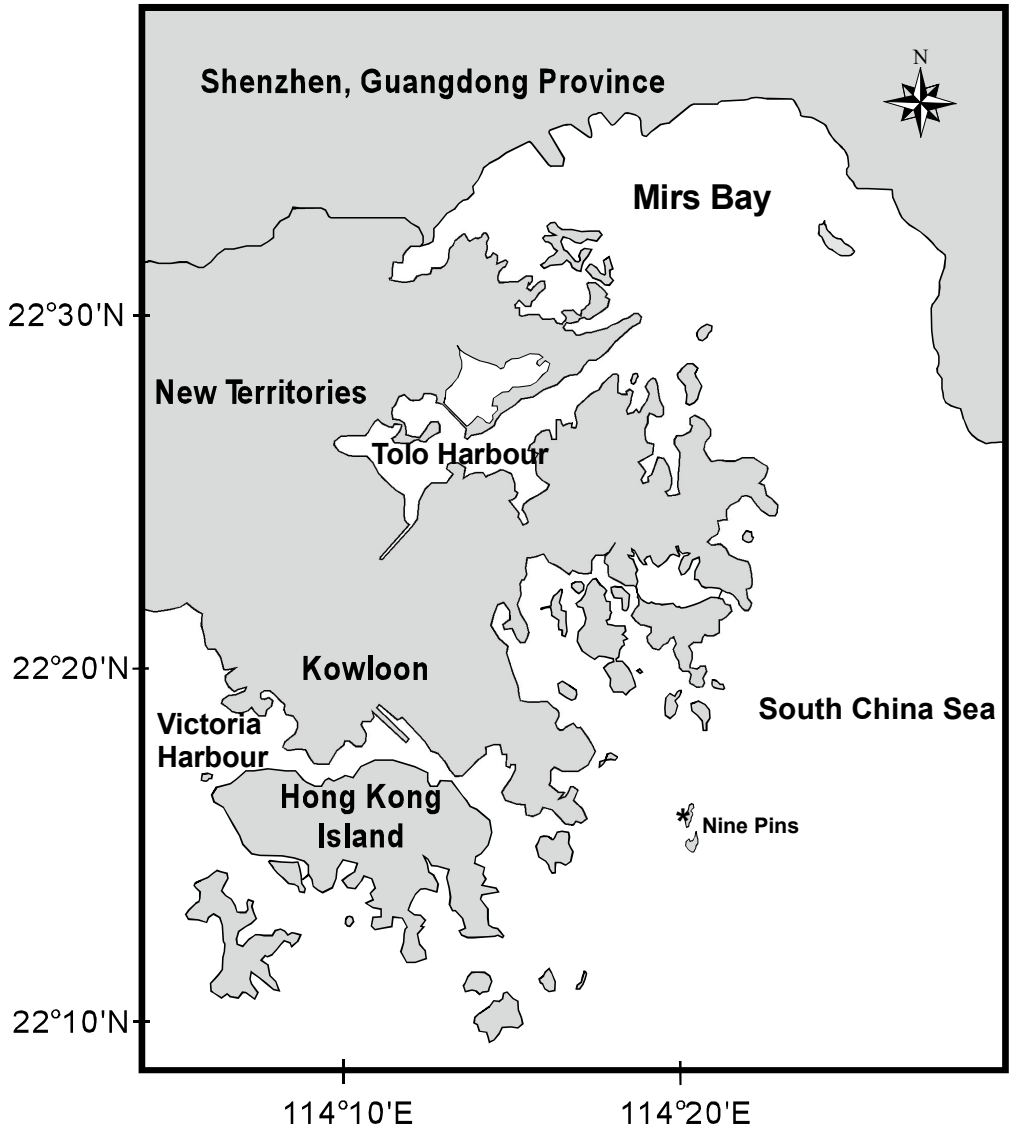

Fig. 1. Map of Hong Kong showing the location of Nine Pins, the site where the sea urchin tagging study was conducted.

several days. Calcein marks on the jaws were examined under an Olympus SZX16 dissecting microscope equipped with an X-Cite 120 automated fluorescent light, and calcein blue marks were observed with a Nikon Eclipse Ti-S compound microscope equipped with a Leica Kubler - Codix fluorescent light. The jaw sizes and seasonal jaw increments were measured using the SPOT 5.0 Advanced Software (Fig. 2).

Jaw length at the time of tagging was defined as the distance from the oral tip to the calcein and/or calcein blue mark (Fig. 2A, B). For tagged sea urchins collected after 376 d, the distance from the calcein mark to the epiphysis junction was considered the 1-year jaw increment (Fig. 2C), the distance from the calcein blue mark to the epiphysis junction was the increment during Season 2 (Fig. 2D), and the distance between calcein and calcein blue marks was the increment during Season 1 (Fig. 2E). In addition, the distance from the calcein mark to the epiphysis was the jaw increment during Season 1 (Fig. 2C) for the sea urchins collected 176 d after tagging.

We applied analysis of covariance (ANCOVA) to compare the mean sea urchin jaw growth among season 1, season 2 and the whole year. The response variable refers to the sea urchin jaw growth and the predictor to the main effects of the season and jaw size at the beginning of the experiment.

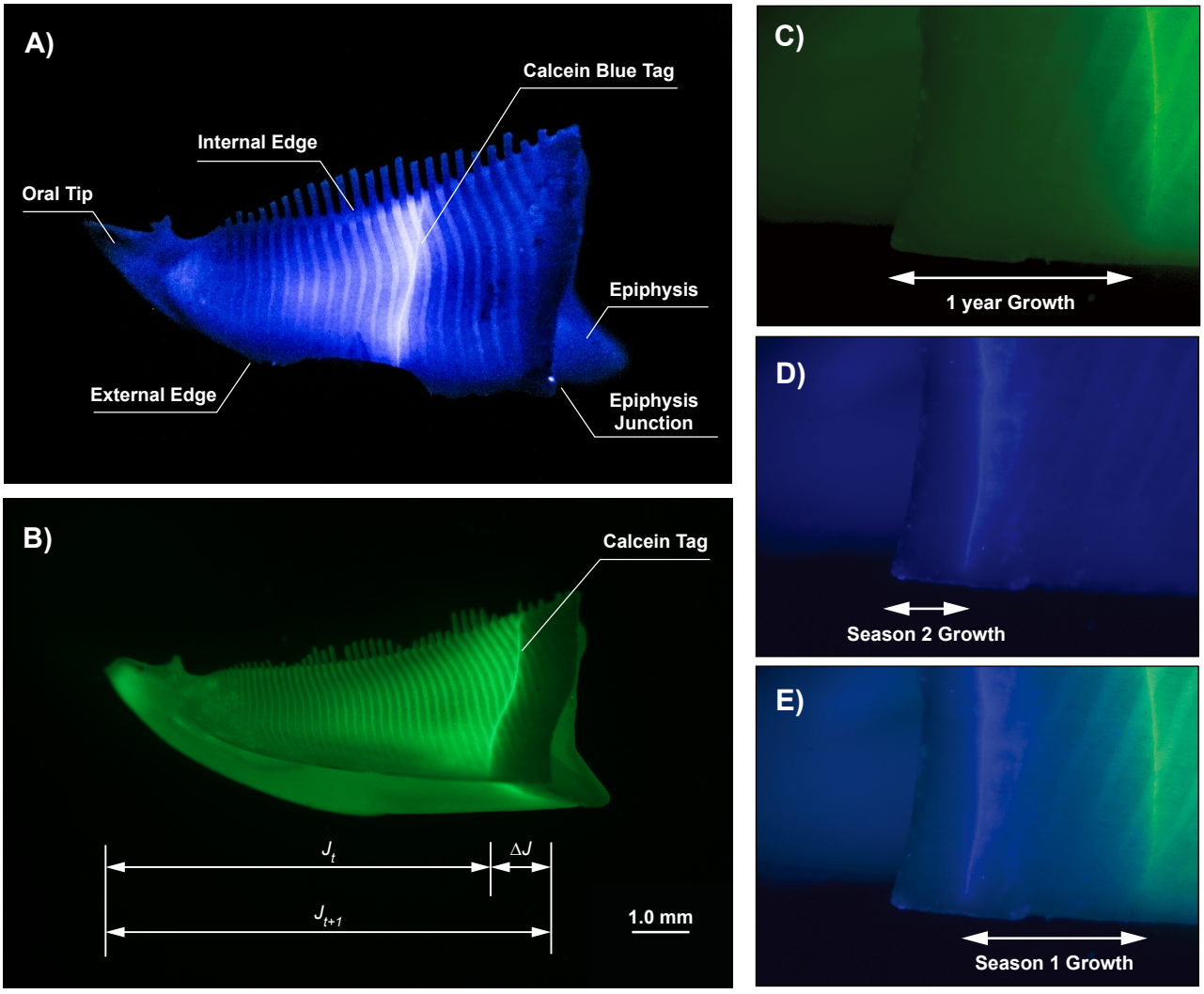

Fig. 2. Jaw of *Heliocidaris crassispina* under ultraviolet illumination with A) calcein blue tag, B) calcein tag. Measurements showing increment of jaw length (ΔJ), jaw length at the time of tagging with calcein (J_t) and jaw length at the time of recapture after one year (J_{t+1}). C) 1-year growth increment (sea urchins collected 376 d after tagging) or Season 1 growth (sea urchins collected 176 d after tagging); marked with calcein. D) Season 2 growth, marked with calcein blue. E) Merged pictures of sequential marking showing seasonal growth over 1 year (376 d); distance between the calcein blue mark and calcein mark is also the Season 1 growth (sea urchins collected 376 d after tagging). A–B: magnification 8.5x. C–E: magnification of 50x.

Modelling

We used a derivation of the von Bertalanffy growth model incorporating seasonal variation (Eqs. 1–4) to estimate the seasonal and non-seasonal (yearly) sea urchin growth (Clasing et al. 1994; Somers 1988):

$$D_t = D_\infty\{1 - e^{-[K(t-t_0)-s(t)+s(t_0)]}\}, \quad (1)$$

where D_t is the expected or average test diameter size at time t, D_∞ the model asymptote for the average length, K the exponential rate of approach to the asymptotic length (growth constant), and t_0 the theoretical age when $D_t = 0$. The term t_0 is lost when a difference equation is formed.

For the von Bertalanffy growth model with seasonal variation in (1), the change in average test diameter size is given by

$$\begin{aligned}\Delta D &= D_{t+\Delta t} - D_t \\ &= (D_\infty - D_t)\{1 - e^{-[K\Delta t-s(t)+s(t+\Delta t)]}\}\end{aligned} \quad (2)$$

where D_t is the original size, $D_{t+\Delta t}$ the size after some time period. $\Delta t = t_2 - t_1$, where t_1 is the time of tagging and and t_2 the time of measurement in Julian date since 1 January (Julian date description, Pritchett 1947). $S(t)$ indicates the seasonal growth pattern of the sea urchin which is defined as

$$S(t) = \frac{CK}{2\pi} sin2\pi(t - t_s), \quad (3)$$

t_s is the parameter that defines the beginning of the sine wave ($-0.5 \le t_s \le 0.5$). The starting point of the oscillation with respect to $t = 0$ is the time between time 0 and the start of the convex portion of the first sinusoidal growth oscillation (*i.e.*, the inflection point). t is the Julian date starting with 1 January as 0 (age at which the length is 0). C is the amplitude of the growth oscillation and corresponds to the proportion of the decrease in growth at the depth of the oscillation (*i.e.*, ''winter''); when $C = 0$, there is no seasonal effect.

The non-seasonal result was obtained by restricting $C = 0$ (no seasonal effect). To estimate the parameters of seasonal and non-seasonal von Bertalanffy models, the parameters of the allometric equation and the parameters of seasonal and non-seasonal models of test diameter, the Broyden-Fletcher-Goldfarb-Shanno (BGFS) algorithm was applied to minimize the sum of squared errors in the R environment version 3.6 (R Development Core Team 2019).

To estimate the original test diameter (TD, mm) from changes in jaw length (J, mm) based on calcein and calcein blue tags, we transformed growth parameters from jaw length (mm) into test diameter (mm) using an allometric relationship between jaw length (mm) and test diameter (mm) established from all tagged sea urchins at the time of recapture with data combined for only the annual tags:

$$TD_t = \alpha J_t^\beta, \quad (4)$$

where α and β are constants calculated using a model II linear regression. Data were log transformed when necessary to meet the assumptions of normality and homogeneity of variance. Normality was tested using the Shapiro-Wilk's test and homogeneity of variances using the Levene test. All analyses were carried out using R version 2.11 R environment version 3.6 (R Development Core Team 2019). The data and script for fitting these models and reproducing the illustrations of this study are available in the supplementary information.

This seasonal model was previously used to estimate the growth of the fishes *Salmo salar*, *Trisopterus esmarkii*, *Sciaenops ocellatus* (Cubillos et al. 2001; Pauly et al. 1992; Porch et al. 2002) and the gastropods *Venus antiqua* and *Nassarius reticulatus* (Chatzinikolaou and Richardson 2008; Clasing et al. 1994). For sea urchins, the seasonal growth was previously estimated with monofilaments and using the size-frequency distributions of *Strongylocentrotus purpuratus* based on monthly collected data over one year (Ebert 2020 1968).

RESULTS

Recapturing sea urchins tagged with calcein and calcein blue

The sea urchins tagged with calcein on 15 February 2012 ranged from 9.1 to 53.2 mm in test diameter (n = 827) and those captured and tagged with calcein blue on 30 August 2012 ranged from 7.2 to 48.3 mm in test diameter (n = 803). The size range of the 50 individuals collected after six months on 30 August 2012 was 27.1 to 44.2 mm in test diameter, and only three carried the calcein tag. Of the 412 individuals collected after one year on 26 February 2013 (18.9 to 42.7 mm in test diameter), 57 were tagged (18.9 to 40.6 mm in test diameter); 34 of these carried the calcein blue tag, 15 the calcein tag, and five both tags. Growth of the latter five individuals was added to growth analysis (or estimates) for Season 1, Season 2 and the whole year. Overall, the percentage of recovered individuals with a fluorochrome tag was 5 to 6% (Fig. 3).

Allometric relationship between demi-pyramid length and test diameter

The following equation describes the allometric relationship between test diameter (*TD*, mm) and jaw length (*J*, mm) (Fig. 4):

$$TD = 10.979J^{0.561} (R^2 = 0.482, n = 57, p < 0.01) \quad (5)$$

Growth

Growth data were obtained from each individual (18.9 to 42.7 mm in test diameter). Sea urchin jaw growth was based on measurements of growth lines of successfully tagged individuals except sea urchins recovered tagged with both fluorochromes after 1-year growth (Fig. 5). After applying ANCOVA, we found that there was no homogeneity of regression slopes as the interaction term was statistically significant at a 5% level, critical value F (2, 61) = 3.14 and *p*-value = 0.0004. The mean growth rate was 3.43 ± 0.73 mm in test diameter (mean ± SE) during Season 1 and 2.23 ± 0.47 mm during Season 2. The mean increase in test diameter over the year was 4.62 mm, less than the sum of the mean growth rate (5.29 mm in test diameter) for

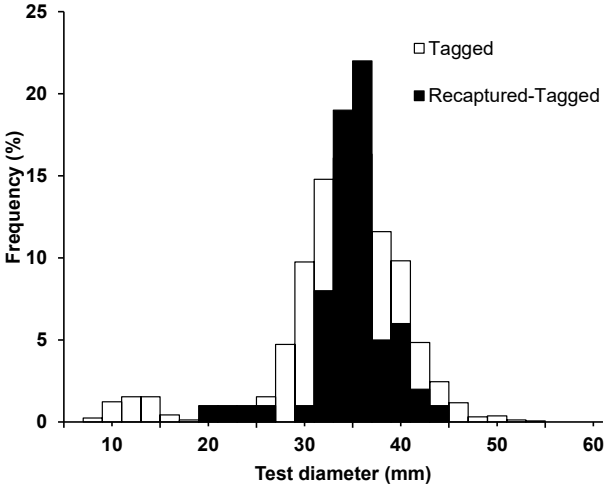

Fig. 3. Size-frequency distribution of *Heliocidaris crassispina* tagged with calcein and calcein blue (*n* = 1630). Solid bars represent recaptured individuals with tags at the end of the experiment (*n* = 57). The mean test diameter (*TD*) of the tagged and recaptured individuals with tags was 33.0 and 34.0 mm, respectively.

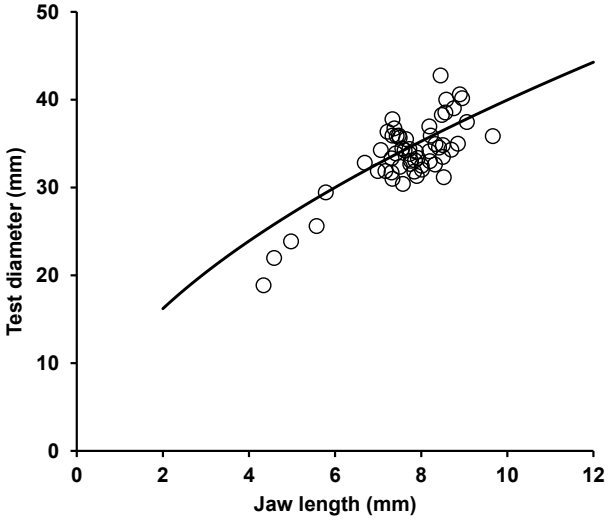

Fig. 4. Allometric relationship between jaw (demi-pyramid) length and test diameter (*TD*) for all recovered *Heliocidaris crassispina* with tags (*n* = 57).

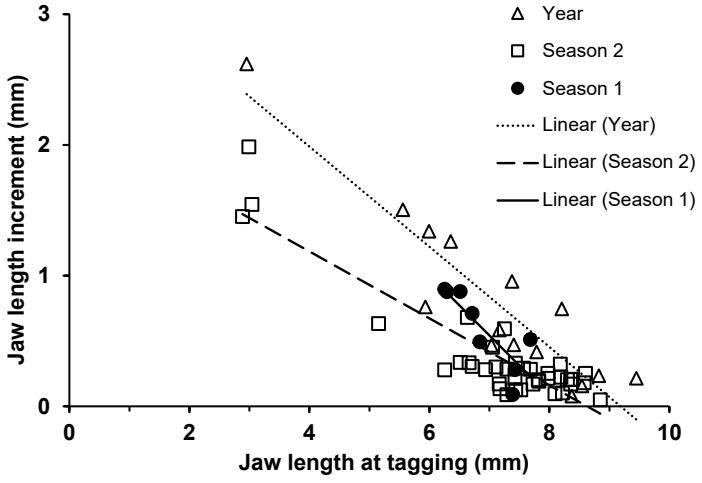

Fig. 5. *Heliocidaris crassispina* annual (*n* = 15) and seasonal growth: Season 1 (*n* = 8) and Season 2 (*n* = 39). Relationship between increment in jaw length after 1 year in the field and jaw length at the time of tagging.

the two seasons.

The parameters of the seasonal and non-seasonal von Bertalanffy growth functions are shown in table 1. The amplitude of the seasonal growth parameter in jaw length is larger than 1, suggesting a difference in the growth of *Heliocidaris crassispina* in the two seasons, with a slower growth rate in Season 2 than Season 1. The parameter *C* measures the size of seasonal variation in growth. In particular, when *C* is greater than 1 or less than -1, the sea urchins will shrink during the non-growth season. When *C* is equal to 0, the growth of sea urchins has no seasonal variation. The calculated asymptotic values for both models were similar. The predicted maximum (asymptotic) size was 38.2 mm in test diameter based on the seasonal growth model and 38.1 mm based on the non-seasonal model. The slopes, represented by the growth constant K (year^{-1}), in the seasonal model was larger than in the non-seasonal model (0.57 vs. 0.42) and t_0 (year) values were also similar. Our estimations indicated that seasonal variations in growth were greatest during the first five years, and less for sea urchins older than seven years. Growth of older sea urchins was similar in different seasons and similar to that predicted by the non-seasonal growth model (Fig. 6).

We estimated the growth of test diameter with and without seasonal variation separately, indicating that test diameter growth curve with and without seasonal variations may not be close to each other. Furthermore, the test diameter growth curve included the randomness due to the estimated allometric equation of jaw length and test diameter.

DISCUSSION

Most studies of sea urchin growth have focused on yearly growth, and have not taken seasonality into account (Ebert 2020). Our sequential labeling technique showed a seasonal change in growth of the purple sea urchin *Heliocidaris crassispina*, illustrating the potential applicability of using two fluorochromes (calcein and calcein blue) to study seasonal growth of sea urchins in the field. Our results show a lower growth rate during September 2012–February 2013 (Season 2) than February–September 2012 (Season 1). Season 2 corresponded to the time when *H. crassispina* had a lower mean gonadosomatic index (2.4) (compared to 4.3 in Season 1), indicating that slower somatic growth coincided with slower gonadal growth in this sea urchin

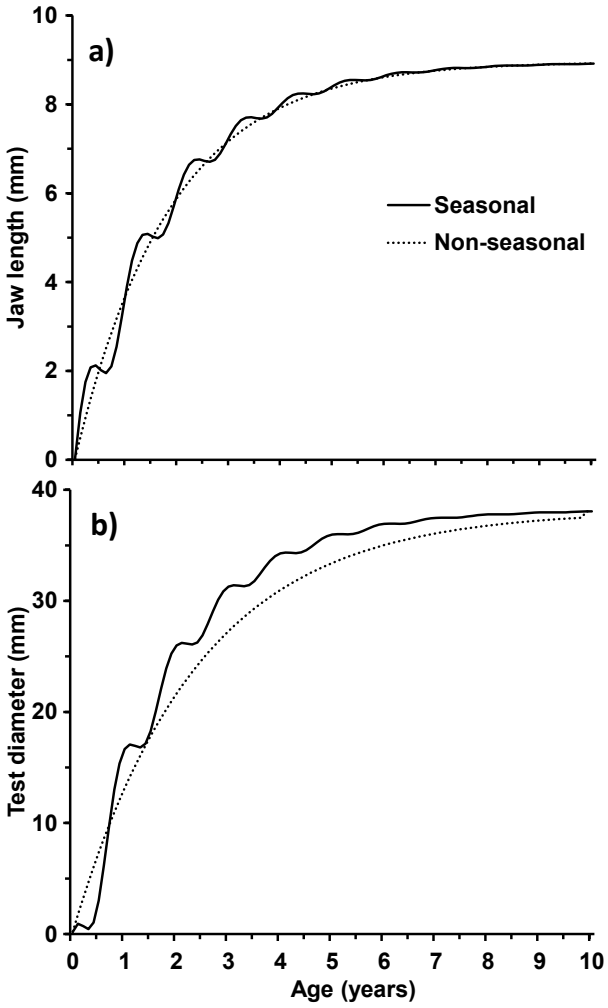

Fig. 6. Seasonal (solid lines) and non-seasonal (dash lines) growth for *Heliocidaris crassispina* described by the von Bertalanffy model (n = 67) for jaw length (a) and test diameter (b).

Table 1. Growth parameters of tagged *Heliocidaris crassispina* collected at Nine Pins describing the von Bertalanffy seasonal and non-seasonal growth for jaw length and test diameter

	Model	D_∞ (mm)	J_∞ (mm)	K (year^{-1})	C	t_s	t_0 (year)
Jaw length	Seasonal	-	8.946	0.569	1.314	-0.021	0.091
	Non-seasonal	-	8.961	0.544	0	0	-0.005
Test diameter	Seasonal	38.191	-	0.568	1.193	-0.295	-0.007
	Non-seasonal	38.125	-	0.417	0	0	-0.013

population (Urriago et al. 2016).

In Hong Kong, a large diversity and an abundance of foliose, filamentous and encrusting algae are present during most of Season 2, and these algae almost completely disappear in Season 1 when the seawater warms up (Kaehler and Kennish 1996). Our results showed that the growth rates of *H. crassispina* were low when algal production was high, which may indicate that there is a lag between the time with high food abundance and the time of somatic and gonadal growth in this species of sea urchin.

Johnson et al. (2013) evaluated the growth of 2-month-old *Strongylocentrotus droebachiensis* sea urchins using sequential tagging in the laboratory by batch-marking via immersion in baths containing fluorochromes tetracycline, calcein, calcein blue, and alizarin complexone. Neither growth nor survival were affected by marking, and the internal marks were subsequently seen in 99% of the tagged individuals after 1 year. Also, 92% adults of *S. droebachiensis* injected with the four fluorochromes recovered after two years showed visible tags. Since internal fluorochrome marks have been detected after 3.9 years in sea urchins (Lamare and Mladenov 2000) and after at least 10 years in corals (Rosenfeld et al. 2003), they have the potential for use in studies of seasonal growth of sea urchins lasting more than one year.

Despite the potential of sequential fluorochrome tagging, in our study the percentage of tagged sea urchins captured was low, which limited the effective use of these data in modelling urchin growth and mortality. A previous study using calcein as the growth marker also found that the tagging success for this species was low (Lau et al. 2011). In this study, we fed the sea urchins in the laboratory during the tagging process and kept them under observation for 24 h after tagging, and increased the number of sea urchins tagged and recaptured, but the results were still not satisfactory. Since food availability may promote calcification and thus tagging success, if the tagging is conducted when food is abundant in the field (*i.e.*, November to March), perhaps the adults could be injected with fluorochrome *in situ* to reduce the potential for physiological stress caused by transporting and rearing sea urchins. Alternatively, the fluorochrome concentration used for injection into adults, and exposure time for bathing juveniles for this species could be increased. The fluorochrome concentration and exposure time we adopted have worked well in a few other species of sea urchins (Ellers and Johnson 2009; Haag et al. 2013; Johnson et al. 2013), but they may not be appropriate for *H. crassispina*. Of course, one has to bear in mind that increasing the fluorochrome concentration and exposure time may cause physiological stress or even death, therefore small scale trials should be conducted in the laboratory in order to come up with an optimal tagging procedure for this species. Moreover, knowledge of whether the urchins could successfully attach to the rocks and the extent to which this sea urchin moves around after tagging would be useful. Although a previous study has indicated that this sea urchin has very limited locomotive capability with a daily activity range confined to 5 m (Freeman 2003), it is not known whether some of the tagged individuals in our study were washed away by waves and currents before they could firmly attach to the rocky substrate. So, collecting tagged *H. crassispina* beyond the tagging zone may increase the chance of capturing tagged sea urchins. Ebert and Russell (1993) evaluated the growth and mortality of the sea urchin *Strongylocentrotus franciscanus* by tagging individuals with tetracycline in the field. They had a similar problem of capturing a small number of tagged sea urchins, suggesting that their low capture rate of tagged animals was due to the movement of the tagged sea urchin out of and untagged *S. franciscanus* into the tag area.

CONCLUSIONS

The present field experiment is the first to provide information on the seasonal growth of the purple sea urchin *Heliocidaris crassispina* using sequential fluorochrome tagging. This species showed slower growth rates during Season 2 compared with Season 1. The slower growth in Season 2 was probably related to the reproductive cycle, which coincided with slow gonadal growth in this sea urchin population. Our study revealed the potential of using sequential fluorochrome tagging to study the seasonal growth of *H. crassispina* as well as other echinoderms and shellfish in the field. We recommend small-scale trials to evaluate the marking success before conducting full-scale experiments.

Acknowledgments: This project was supported by Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0404, L2019005), the University Grants Committee (grant number HKBU261210) and KMITL (grant number KREF145902). We thank Gray Williams of the Swire Institute of Marine Science of the University of Hong Kong for encouragement and support, boat captains Ming Cheung and Hong Cheung for assistance in the field, Eric Goh Zhe, Lai Kean Sim, Juan Carlos Astudillo and Magdalena Taczkowska for assistance in the field and laboratory, and John H. Himmelman for helpful comments on the manuscript.

Authors' contributions: M.Sc. Juan Diego Urriago Suarez: Methodology, data acquisition, software, formal analysis, formal analysis, writing - original draft, writing - review and editing, visualization. Dr. Jane C.Y. Wong: Data acquisition. Dr. Gilbert Lui: Methodology, software. Dr. Clement P. Dumont: Conceptualization, methodology, writing - review and editing, supervision. Dr. Jian-Wen Qiu: Conceptualization, methodology, supervision, funding acquisition, writing - review and editing. Dr. Monthon Ganmanee: Supervision, funding acquisition.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no competing interests.

Availability of data and materials: The data generated and analyzed during the current study are available from the supplementary materials.

Consent for publication: All the authors consent to the publication of this manuscript.

Ethics approval consent to participate: Not applicable.

REFERENCES

Agatsuma Y. 2007. Ecology of *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus*, and *Anthocidaris crassispina*. *In*: JM Lawrence (ed) Edible sea urchins: biology and ecology, vol 2. Elsevier, pp. 459–472. doi:10.1016/S0167-9309(07)80086-X.

Agatsuma Y. 2013. *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus*, and *Heliocidaris crassispina*. *In*: ML John (ed) Sea urchins: biology and ecology, vol 38. Elsevier, pp. 461–473. doi:10.1016/B978-0-12-396491-5.00030-7.

Campbell DA, Pantazis P, Kelly MS. 2001. Impact and residence time of oxytetracycline in the sea urchin, *Psammechinus miliaris*, a potential aquaculture species. Aquaculture **202:**1–2. doi:10.1016/S0044-8486(01)00600-7.

Chatzinikolaou E, Richardson C. 2008. Population dynamics and growth of *Nassarius reticulatus* (Gastropoda: Nassariidae) in Rhosneigr (Anglesey, UK). Mar Biol **153(4):**605–619. doi:10.1007/s00227-007-0835-5.

Chiu ST. 1987. Aspects of the ecology of *Anthocidaris crassispina* (Echinodermata: Echinoidea) in Hong Kong. Dissertation, University of Hong Kong.

Chiu ST. 1990. Age and growth of *Anthocidaris crassispina* (Echinodermata, Echinoidea) in Hong Kong. Bull Mar Sci **47(1):**94–103.

Clasing E, Brey T, Stead R, Navarro J, Asencio G. 1994. Population dynamics of *Venus antiqua* (Bivalvia: Veneracea) in the Bahía de Yaldad, Isla de Chiloé, Southern Chile. JEMBE **177(2):**171–186. doi:10.1016/0022-0981(94)90235-6.

Cubillos LA, Arcos DF, Bucarey DA, Canales MT. 2001. Seasonal growth of small pelagic fish off Talcahuano, Chile (37°S, 73°W): a consequence of their reproductive strategy to seasonal upwelling? Aquat Living Resour **14(2):**115–124. doi:10.1016/S0990-7440(01)01112-3.

Ebert TA. 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. Ecology **49(6):**1075–1091. doi:10.2307/1934491.

Ebert TA. 1988. Calibration of natural growth lines in ossicles of two sea urchins, *Strongylocentrotus purpuratus* and *Echinometra mathaei*, using tetracycline *In*: Echinoderms: Proceedings of the 6th International Echinoderm Conference, Rotterdam, pp. 435–444.

Ebert TA. 1999. Plant and animal populations: methods in demography. Academic Press, San Diego; Toronto.

Ebert TA. 2013. Growth and survival of postsettlement sea urchins. *In*: ML John (ed) Sea urchins: biology and ecology, vol 38. Elsevier, pp. 83–117. doi:10.1016/B978-0-12-396491-5.00007-1.

Ebert TA. 2020. Growth and survival of postsettlement sea urchins. *In*: JM Lawrence (ed) Sea Urchins: Biology and Ecology. Developments in Aquaculture and Fisheries Science, vol 43. Elsevier, pp. 95–145. doi:10.1016/b978-0-12-819570-3.00007-x.

Ebert TA, Russell MP. 1993. Growth and mortality of subtidal red sea urchins (*Strongylocentrotus franciscanus*) at San Nicolas Island, California, USA: problems with models. Mar Biol **117(1):**79–89. doi:10.1007/BF00346428.

Ellers O, Johnson AS. 2009. Polyfluorochrome marking slows growth only during the marking month in the green sea urchin *Strongylocentrotus droebachiensis*. Invertebr Biol **128(2):**126–144. doi:10.1111/j.1744-7410.2008.00159.x.

EPD. 2012. Marine water quality in Hong Kong in 2012. Hong Kong: Environmental Protection Department (EPD) of HKSAR Government.

Freeman SM. 2003. Size-dependent distribution, abundance and diurnal rhythmicity patterns in the short-spined sea urchin *Anthocidaris crassispina*. Estuar Coast Shelf Sci **58(4):**703–713. doi:10.1016/S0272-7714(03)00134-3.

Gage J. 1991. Skeletal growth zones as age-markers in the sea urchin *Psammechinus miliaris*. Mar Biol **110(2):**217–228. doi:10.1007/BF01313707.

Haag N, Russell MP, Hernandez JC, Dollahon N. 2013. Assessing fluorochrome-staining efficacy in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller, 1776). Cah Biol Mar **54:**625–631. doi:10.21411/CBM.A.EFE968FB.

Johnson AS, Salyers JM, Alcorn NJ, Ellers O, Allen JD. 2013. Externally visible fluorochrome marks and allometries of growing sea urchins. Invertebr Biol **132(3):**251–269. doi:10.1111/ivb.12024.

Kaehler S, Kennish R. 1996. Summer and winter comparisons in the nutritional value of marine macroalgae from Hong Kong. Bot Mar **39(1-6):**11–18. doi:10.1515/botm.1996.39.1-6.11.

Kalvass PE, Hendrix JM, Law PM. 1998. Experimental analysis of 3 internal marking methods for red sea urchins. Calif Fish Game **84(2):**88–99.

Kobayashi S, Taki J. 1969. Calcification in sea urchins: I. A Tetracycline investigation of growth of the mature test in *Strongylocentrotus intermedius*. Calc Tiss Res **4:**210–223. doi:10.1007/BF02279124.

Lamare MD, Mladenov PV. 2000. Modelling somatic growth in the sea urchin *Evechinus chlorotichus* (Echinoidea: Echinometridae). JEMBE **243:**17–43. doi:10.1016/S0022-0981(99)00107-0.

Lau DCC, Dumont CP, Lui GCS, Qiu JW. 2011. Effectiveness of a small marine reserve in southern China in protecting the harvested sea urchin *Anthocidaris crassispina*: A mark-and-recapture study. Biol Conserv **144(11):**2674–2683. doi:10.1016/j.biocon.2011.07.027.

Observatory HK. 2013. Monthly Mean Sea Surface Temperatures. Hong Kong Observatory, Government of the Hong Kong Special Administrative Region. http://www.weather.gov.hk/en/cis/normal/1981_2010/seatemp.htm#. Accessed 23 June 2014.

Pauly D, Soriano-Bartz M, Jarre-Teichmann A, Moreau J. 1992. A new model accounting for seasonal cessation of growth in fishes. Aust J Mar Freshw Res **43:**1151–1156. doi:10.1071/MF9921151.

Pautke C, Vogt S, Tischer T, Wexel G, Deppe H, Milz S, Schieker M, Kolk A. 2005. Polychrome labeling of bone with seven different fluorochromes: Enhancing fluorochrome discrimination by spectral image analysis. Bone **37(4):**441–445. doi:10.1016/j.bone.2005.05.008.

Porch CE, Wilson CA, Nieland DL. 2002. A new growth model for red drum (*Sciaenops ocellatus*) that accommodates seasonal and ontogenic changes in growth rates. Fish Bull **100(1):**149–152.

Pritchett WK. 1947. Julian Dates and Greek Calendars. Class Philol **42(4):**235–243. doi:10.1086/363049.

R Development Core Team. 2019. R: a language and evironmental for statistical computing. R Foundation for statistical computing, Vienna, Austria.

Rosenfeld M, Yam R, Shemesh A, Loya Y. 2003. Implication of water depth on stable isotope composition and skeletal density banding patterns in a *Porites lutea* colony: results from a long-term translocation experiment. Coral Reefs **22(4):**337–345. doi:10.1007/s00338-003-0333-2.

Russell MP, Urbaniak LM. 2004. Does calcein affect estimates of growth rates in sea urchins? *In*: Proceedings of the 11th International Echinoderm Conference, Rotterdam, pp. 53–57.

Somers I. 1988. On a seasonally oscillating growth function. Fishbyte **6(1):**8–11.

Stefánsson G, Kristinsson H, Ziemer N, Hannon C, James P. 2017. Markets for sea urchins: a review of global supply and markets. Internal Matis report: Skýrsla Matís 10–17.

Stuart AJ, Smith DA. 1992. Use of the Fluorochromes Xylenol Orange, Calcein Green, and Tetracycline to Document Bone Deposition and Remodeling in Healing Fractures in Chickens. Avian Dis **36:**447–449. doi:10.2307/1591527.

Urriago JD, Wong JCY, Dumont CP, Qiu JW. 2016. Reproduction of the short-spined sea urchin *Heliocidaris crassispina* (Echinodermata: Echinoidea) in Hong Kong with a subtropical climate. Reg Stud Mar Sci **8:**445–453. doi:10.1016/j.rsma.2016.03.005.

Yatsuya K, Nakahara H. 2004. Density, growth and reproduction of the sea urchin *Anthocidaris crassispina* (A. Agassiz) in two different adjacent habitats, the *Sargassum* area and *Corallina* area. Fish Sci **70(2):**233–240. doi:10.1111/j.1444-2906.2003.00796.x.

Supplementary Materials

Fig. S1. A) *Heliocidaris crassispina* (> 20 mm in test diameter) tagging by injecting 2 ml of calcein solution into the coelom (500 ppm of calcein in natural filtered seawater). B) Outdoor tanks with a continuous flow of sea water where sea urchins were fed for 3 d after tagging. (download)

Fig. S2. *Heliocidaris crassispina* late juveniles (< 20 mm in test diameter) bathed in the fluorochrome calcein solution (500 ppm of calcein in natural filtered seawater). Sea urchins were fed in aerated seawater. (download)

DataSet. The data below were used to create figures 5 and 6 and parameters in table 1. Yearly data (t = 1 and dt = 1), Season 1 data (t = 1.5 and dt = 0.5), Season 2 data (t = 1 and dt = 0.5, initial jaw size in mm (JS_start), final jaw size in mm (JS_end), final test diameter in mm (TD_end). (download)