African Journal of Biotechnology Vol. 9(44), pp. 7560-7563, 1 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1332
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Test and gonad characteristics in different genders of cultivated sea urchins (*Strongylocentrotus intermedius*, Agassiz): First insight into sexual identification

Chong Zhao, Weijie Zhang, Yaqing Chang* and Pingjin Liu

Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Ocean University, Dalian-116023, China.

Accepted 8 October, 2010

Ten test and gonad characters in both genders of cultured sea urchins (*Strongylocentrotus intermedius*, Agassiz) were investigated for sexual identification and sex ratio. Both test (test height-diameter ratio) and gonad characters (gonad index, gonad color and gonad moisture content) were significantly different between male and female sea urchins. For the first time, we report that cultivated female sea urchins are flatter than males. This could provide some new insight into the sexual identification of *S. intermedius*. The sex ratio of cultured sea urchins kept 1:1 at the density of 5 × 10^3g/m^3, suggests that land-based aquaculture of sea urchins at a density of 5 × 10^3g/m^3 can keep the balanced equilibrium on the sex ratio.

Key words: *Strongylocentrotus intermedius*, test height-diameter ratio, sexual identification, sex ratio in aquaculture.

INTRODUCTION

The sea urchin *Strongylocentrotus intermedius* was a commercially important species originally found off the coast from Hokkaido of Japan and Far East Russia. It was introduced from Japan to China in 1989 (Chang et al., 2004). Gonads of *S. intermedius* have great commercial potential for its excellent quality (Chang et al., 2004). In China, the annual production of roe of *S. intermedius* amounts to 200 tons (Ding et al., 2007). Gonad characteristics have been largely investigated for it is the only edible part in sea urchins (Phillip et al., 2009). To the best of our knowledge, however, the relationship of gonad and test characteristics has scarcely been reported. Sex study is an important research area in aquaculture. Aqua-farmers urgently need to be able to identify the sex of sea urchins before using them for spawning. In China, the current breeding methods without sexual identification definitely increase the cost. This suggests that an effective method for sexual identification would be commercially beneficial. However, no information that permits identification of sex before spawning in sea urchins is available.

The sex ratio is of importance to maintain the ecological and genetic balance of species. A number of studies have been done to identify unusual sex ratio and probable factors on sea urchin populations in the field (Brewin et al., 2000; Coppard and Campbell, 2005; Gianguzza et al., 2008; Gonor, 1973; Lamare and Stewart, 1998; Levitan, 2002; McPherson, 1965). However, to our knowledge, no information is available on the sex ratio of sea urchins in aquaculture, although increasing commercial demand has resulted in a great interest in their aquaculture. This lack of knowledge greatly limits our understanding of cultivated *S. intermedius*. Consequently, we were strongly motivated to investigate test and gonad characteristics of different genders of cultivated sea urchins, *S. intermedius*. It could provide some new insight into the sexual identification and sex ratio.

*Corresponding author. E-mail: yaqingchang@hotmail.com. Tel: +86 411 84762131.

Abbreviations: GI, Gonad index; **GMC,** gonad moisture content; **HDR,** test height-diameter ratio; **TH,** test height; **TD,** test diameter; **BW,** body weight; **HWR,** test height-body weight ratio; **DWR,** test diameter-body weight ratio; **GW,** gonad wet weight.

MATERIALS AND METHODS

Twenty full-sib families of sea urchins were produced and cultured

Table 1. The mean (±SD) of the variables in different genders of *S. intermedius*.

Gender		TH	TD	BW	*L**	*a**	*b**	GW	GI	GMC	HDR	HWR	DWR
Male													
	Mean	16.42	37.19	22.35	78.69	22.14	23.31	2.73	11.92	71.01	0.44	0.82	1.87
	N	268	268	268	266	266	266	268	268	268	268	268	268
	SD	2.77	5.48	9.12	4.22	7.47	6.53	1.48	3.52	8.08	0.03	0.23	0.55
Female													
	Mean	16.31	37.51	23.17	75.50	31.66	30.30	2.70	11.27	68.96	0.43	0.81	1.88
	N	264	264	264	262	262	262	264	264	264	264	264	264
	SD	3.00	6.17	10.35	3.16	5.53	6.65	1.55	3.42	9.76	0.03	0.29	0.68
Total													
	Mean	16.37	37.35	22.75	77.11	26.86	26.78	2.72	11.60	69.99	0.44	0.82	1.87
	N	532	532	532	528	528	528	532	532	532	532	532	532
	SD	2.89	5.83	9.75	4.05	8.12	7.45	1.51	3.48	9.01	0.03	0.26	0.62

TH, Test height (mm); TD, test diameter (mm); BW, body weight (g); *L**, lightness; *a**, redness; *b**, yellowness; GW, gonad wet weight (g); GI, gonad index; GMC, gonad moisture content; HDR, test height-diameter ratio; HWR, test height-body weight ratio; DWR, test diameter-body weight ratio.

in the Key Laboratory of Mariculture, Dalian Ocean University, at an aquaculture density of 5×10^3 g/m^3 and feeding the kelp *Laminaria japonica*. After 19 months of culture, 30 individuals were randomly selected from each family for experiments in June, 2009. Both test and gonad characters were analyzed and compared between genders. Test diameter and height of sea urchins were measured using digital calipers. Their weights were measured with an electronic balance. After the measurements, sea urchins were dissected without damaging the gonads. The entire gonads were then removed and weighed. Gender was identified by colors of gametes released from the gonads. One gonad was dried at 60℃ for 72 h and then weighed. Gonad color readings were taken using PANTONE Color Cue® 2 to measure the *L** *a** *b** (*L**= lightness, *a** = redness and *b** = yellowness). Other variables measured and analyzed throughout the experiment were:

$$GI = \frac{gw}{wb} \times 100\%$$

Where GI = gonad index, gw = wet weight of gonads, and wb = body weight.

$$GMC = \frac{wg'}{dg'} \times 100\%$$

Where GMC = gonad moisture content, wg' = wet weight of the gonad, and dg' = dried weight of the gonad.

$$HDR = \frac{th}{td}$$

Where HDR = test height-diameter ratio, th = test height, and td = test diameter.

$$HWR = \frac{th}{wb}$$

Where HWR = test height-body weight ratio, th = test height, and wb = body weight.

$$DWR = \frac{td}{wb}$$

Where DWR = test diameter-body weight ratio, td = test diameter, and wb = body weight.

The sex ratio was calculated as the number of male sea urchins divided by the number of females.

All variables were calculated using Excel for Windows XP. All percentage data was arcsine-transformed prior to the analysis. T-test was then performed with the Statistical Package for the Social Sciences (SPSS) 16.0 statistical software to check for equality of variances and differences of all test and gonad characters between genders. A probability level of $P < 0.05$ was considered statistically significant. To examine whether the sex ratio deviated from 1:1, a chi-square test was carried out, where $P < 0.05$ was considered statistically significant.

RESULTS

All variables were investigated in both genders (Table 1). The average test diameter of males and females was 37.19 and 37.51 mm, respectively. Body weights were also similar between genders, with the averages of 22.35 and 23.17 g, respectively. Significant differences were found in test (test height-diameter ratio) and gonad characters (gonad index, color and moisture content) between males and females ($P < 0.05$, Table 2). According to mean lightness (*L**), redness (*a**) and yellowness (*b**) readings, female sea urchins had significantly higher *a** and *b** but lower *L** than males ($P < 0.05$). Male sea urchins, on the other hand, showed a significantly higher gonad index (GI), gonad moisture content (GMC) and test height-diameter ratio (HDR) ($P < 0.05$). However, no

Table 2. T-test of variables in both genders of *S. intermedius*.

Variable	Levene's test for equality of variances		T-test for equality of means variance equal or not		
	F	Significance	t	df	Significance (2-tailed)
TH	1.313	0.252	0.436	530	0.663
TD	4.326	0.038	-0.630	520.925	0.529
BW	5.021	0.025	-0.971	519.680	0.332
*L**	38.646	0.000	9.822	490.946	0.000
*a**	6.979	0.008	-16.660	488.569	0.000
*b**	12.209	0.001	-12.185	525.408	0.000
GW	0.631	0.427	0.171	530	0.864
HDR	0.730	0.393	2.759	530	0.006
HWR	4.558	0.033	0.254	503.891	0.800
DWR	4.402	0.036	-0.286	502.584	0.775
GI	0.068	0.795	2.150	530	0.032
GMC	0.459	0.498	2.475	529	0.014

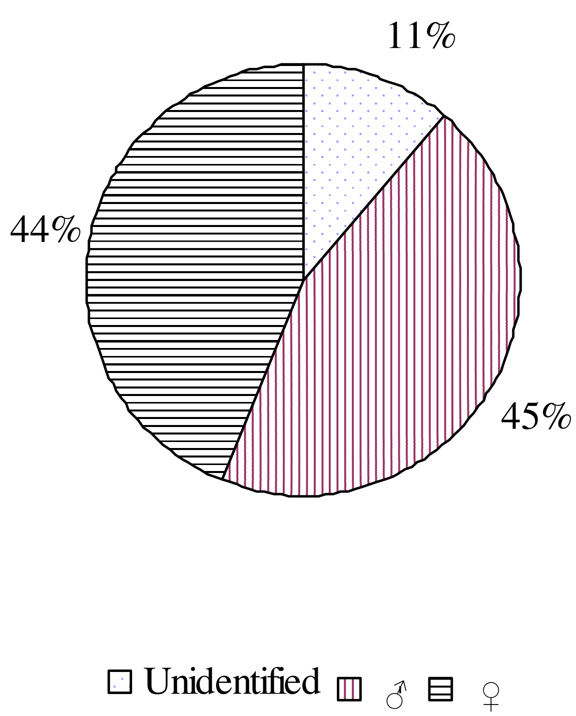

Figure 1. Sex ratio of sampled male and female urchins. ♂: male; ♀: female.

significant difference was found in test height (TH), test diameter (TD), body weight (BW), test height-body weight ratio (HWR), test diameter-body weight ratio (DWR) and gonad wet weight (GW) between genders (P > 0.05).

268 males and 264 females were identified from all 600 samples. The sex of 68 individuals (11%) could not be determined. The chi-square test indicates that the sex ratio of these individuals was not significantly different from the expected ratio (1:1) (P > 0.05) (Figure 1).

DISCUSSION

In sea urchins, color is an important roe trait (Symonds et al., 2009). In the present study, male urchins had higher *L** but lower *a** and *b** than females on color performance. It might be due to released gametes of sea urchins. This result agrees with Phillips et al. (2009) who found that ovaries were rated significantly higher than testes in the sea urchin *Evechinus chloroticus*. Phillips et al. (2009) found no difference in test diameter, wet weight, gonad wet weight and gonad index between male and female urchins. This is partly in agreement with the present study in test diameter, body weight and wet weight of the gonad. However, beyond our expectation, we found that male urchins had significantly higher GI and GMC than females in our study. These disagreements are probable due to differences between the species.

Sexual identification is essential in artificial breeding programs of marine animals (Chang, 2007). The original motivation of the present study was to find some information to identify the gender of sea urchins. We measured a number of test characters and found that males had significantly higher HDR than females (P < 0.01). This suggests that cultured female sea urchins are flatter in the reproductive season. This is the first report on different test characteristics in male and female sea urchins and could provide some new insights into sex identification in *S. intermedius*. Although, the mechanism remains unknown, there may be a link between the gender related and test-shaping genes. Regarding our original aim, however, the present study is far from being a practical method for sexual identification of sea urchins. Further studies will be necessary to provide a practical method for sea urchin aquaculture.

In sea urchins, most related studies have focused on natural populations (Brewin et al., 2000; Coppard and

Campbell, 2005; Gianguzza et al., 2008; Gonor, 1973; Lamare and Stewart, 1998; Levitan, 2002; McPherson, 1965). In wild populations, sea urchins always keep the sex ratio of 1:1 to enhance the stability of the population.

It has also been reported that the ratio can be affected by different mating behaviors (McCarthy and Young, 2002) and different levels of natural predation (Gianguzza et al., 2008) between genders. However, it still remains unknown whether the sex ratio of sea urchins deviates from the 1:1 in aquaculture. To our knowledge, the present study is the first investigation of the sex ratio of cultured sea urchins. Our results showed that the sex ratio of cultivated sea urchins is also 1:1, indicating that the land-based aquaculture of sea urchins at a suitable density can keep the balanced equilibrium on the sex ratio.

In conclusion, we investigated the test and gonad characteristics of different genders of cultivated sea urchins. Here we report: 1) *S. intermedius* has different test and gonad characteristics between genders; 2) the test height-diameter ratio could provide some new insight for sexual identification of *S. intermedius* and 3) the sex ratio of sea urchins *S. intermedius* does not deviate from 1:1 in aquaculture at a density of 5×10^3 g/m^3. The present study could be of some help to provide new insight into sea urchin aquaculture.

ACKNOWLEDGEMENTS

This work was supported by the Chinese National 863 Project (2006AA10A411) and National Natural Science Foundation of China (30972269). We thank Bo Zhang, Jing Zhang, Binglong Zhang and Yanping Ma for sample collections. We are grateful to Prof. John Lawrence for providing many useful editorial suggestions.

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