The 11th International Abalone Symposium

Poua te mana o Pāua ki te tai, kia whakaika te moana!

厦门大学底栖生物学实验室

Biology of Marine Benthic Invertebrates Group

Research on the autotriploid and allotriploid abalone induction and phenotypic traits assessment

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Contents

01 Background

Background

Food sources

Jewellery

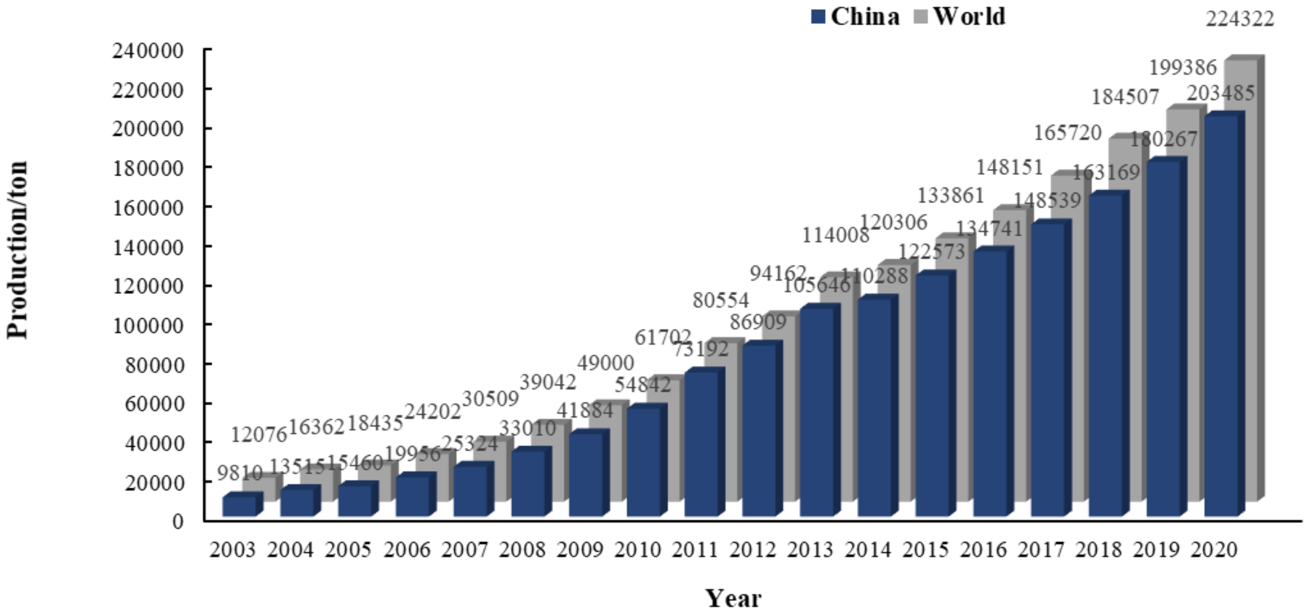
■ **Status of industry**

- Total production in China:
  218,700 tons (2021)

- Proportion of global production:
  more than 90%

- **China is the largest producer and consumer of abalone worldwide**

- **Major aquaculture abalone species in China :**
  Pacific abalone (*Haliotis discus hannai*)



Pacific abalone

Haliotis discus hannai

Background

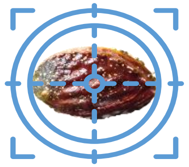
■ Genetic breeding of abalone

Mainly based on the traditional breeding techniques of selection and crossbreeding

Meat quality

Food conversion rate

Gonad development



Shell color

Growth

Resistance

Intraspecific hybridization

"Dalian No.1" | Fast growth and strong resistance
"Dongyou No.1" | Higher survival rate

Interspecific hybridization

Xipan Abalone | Heat resistance
Lvpan Abalone | Heat resistance and fast growth

Selection

"Xunshan No.1" | Cold resistance

The negative effects of sexual maturation

One-year old sexual precocity

Arrested growth

Stress responding deficiency according to trade-off

New issues in China abalone aquaculture

Traditional breeding techniques

Arrested growth or mortality

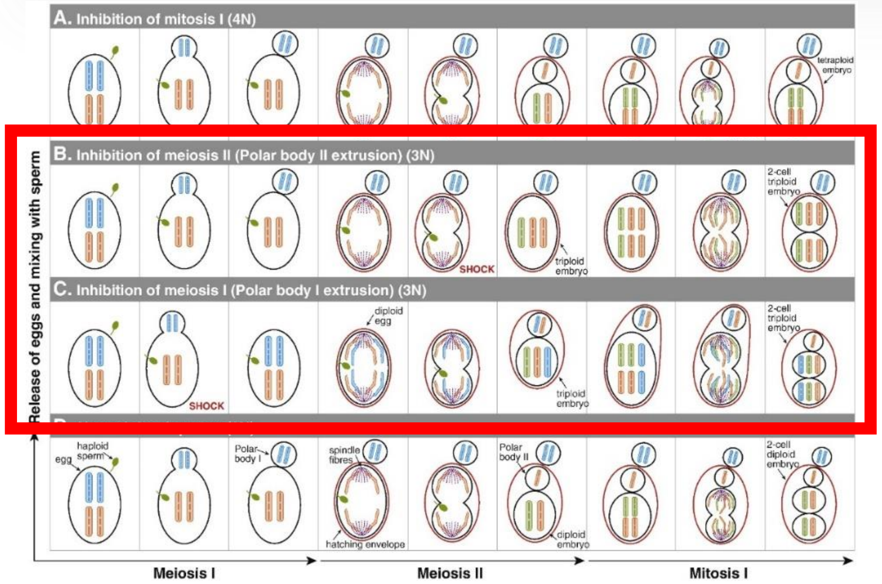
(sexual precocity and spawning)

Background

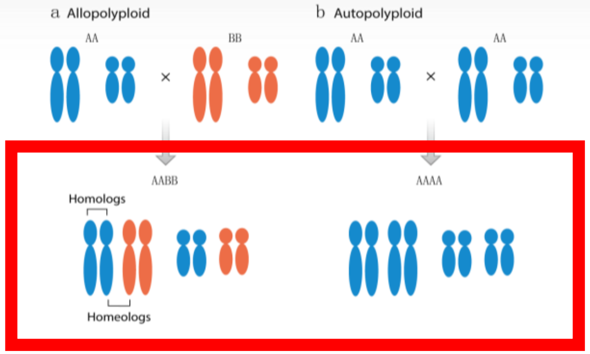
■ Chromosome manipulation—Shellfish triploid breeding

➢ **Triploid**: **Fast growth, sterility or infertile, avoid the growth retardation or mortality of cultured shellfish caused by precocity or spawning.** (Guo et al. 2009; Song et al. 2010) .

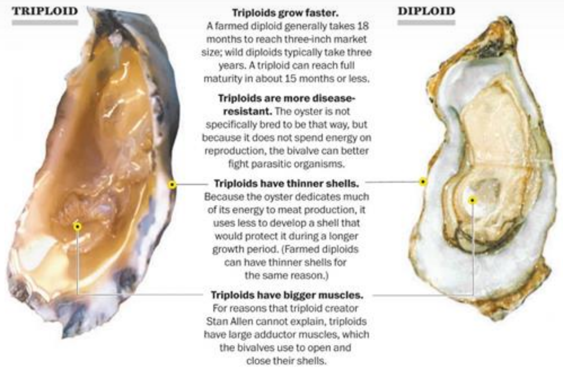
- **Methods**: Physical treatments, chemical treatments and crossing diploids with tetraploids
- **Principles**: Inhibiting polar body I (PB1), inhibiting polar body II (PB2)
- **Types**: **Autotriploid**, **Allotriploid**
- **Species**: more than 20 molluscan species, including oysters, clams, scallops, mussels, pearl oysters and abalones



Process of inducing triploidy (Sellars et al. 2010)



Two types of polyploids (Yoo et al. 2014)



The **advantage of triploidy in the Pacific oyster** is **14% - 159%**. All-triploid of Pacific oysters and Portuguese oysters produced by tetraploidy have been commercialized in the United States, Mexico, Australia, and China (Yang et al. 2018, 2019).

Background

Triploid breeding of abalone

Induction of triploid abalone

H. discus hannai: Induction, growth, thermal tolerance

H. diversicolor diversicolor: Induction

Induction of triploid abalone growth and gonadal development in triploid

H. rubra: Growth, gonadal development

H. laevigata: Induction, growth, gonadal development

H. rufescens (♀) × *H. fulgens* (♂): Induction

H. discus hannai: Induction

1985 | 1990 | 1995 | 2000 | 2005 | 2010 | 2015 | 2020

The triploid ratio of *H. discus hannai*: < 80%

Induction of triploid abalone and larvae growth

H. discus hannai: Induction, growth, gonadal development

H. midae: Induction, larvae growth

H. diversicolor supertexta: Induction

H. rufescens: Induction larvae growth

H. asinine: Induction

H. rubra: Induction, larvae growth and survival

H. discus hannai: Cytogenetic analysis, transcriptome expression of heat-stress and hypoxia

H. midae: Growth, gonadal development

The number of researches decreased

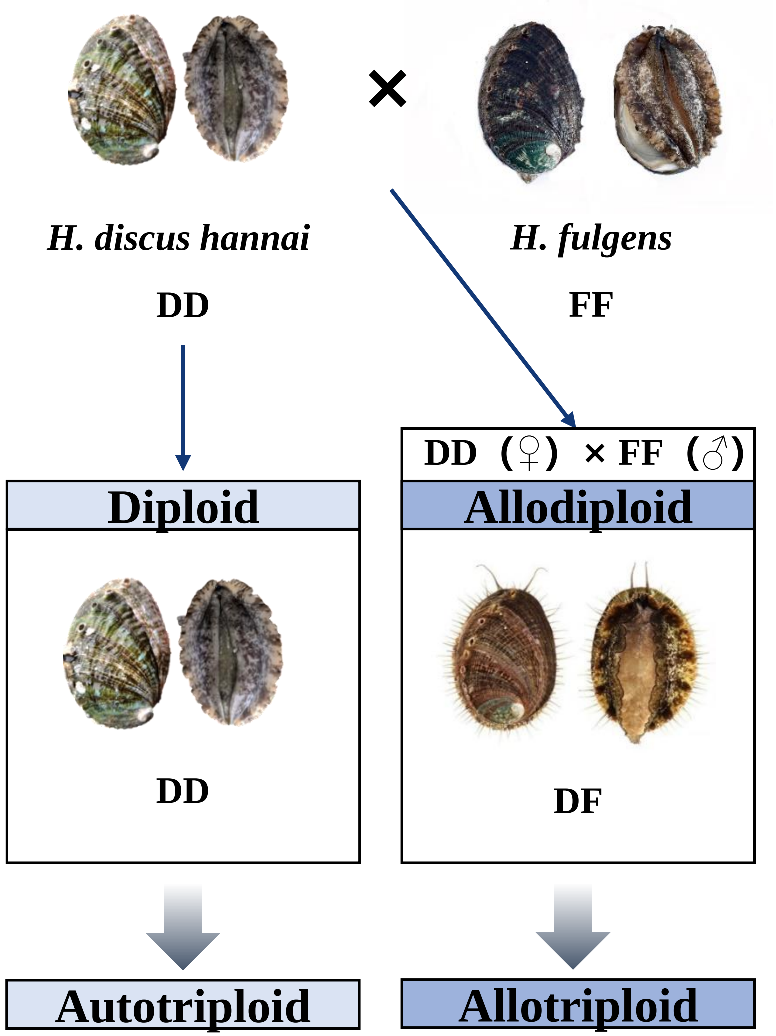
Triploid abalone: Fast growth, sterility or infertile

Problems：

- **The results of triploid production were inconsistent.**
- **Allotriploid adult have not been obtained.**
- **Partial assessment did not identify the ploidy status of individuals within the treatment population.**

Triploid breeding of abalone ✕ Prodution

Background



Screening of **optimal induction conditions of the chemical induction** for autotriploid (*H. discus hannai*) and allotriploid (*H. discus hannai* ♀ × *H. fulgens* ♂).

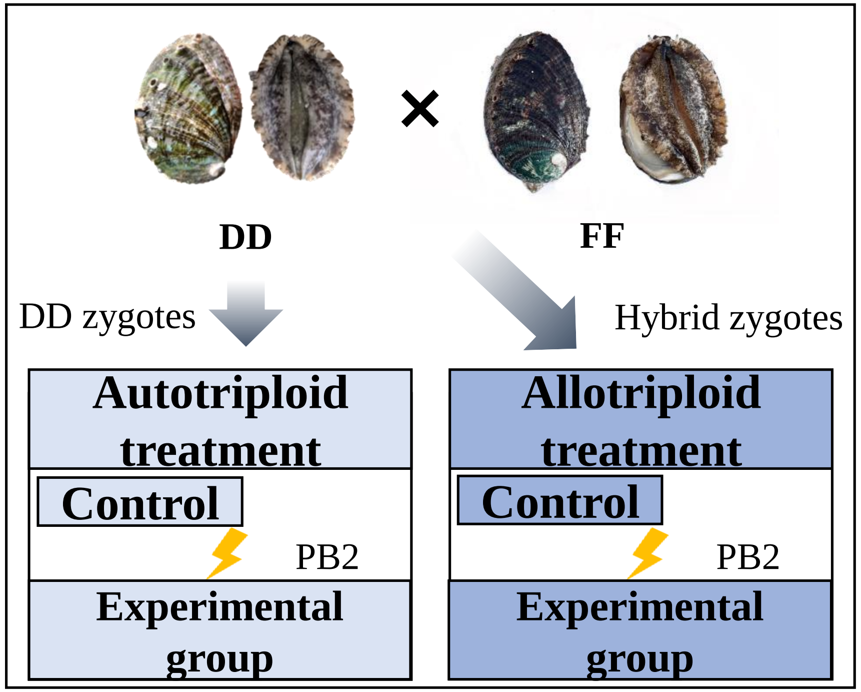
Establishing stable and high efficiency triploid breeding technology of abalone

Assessing the **phenotypic traits of autotriploid and allotriploid on a sea-based suspended system** in a subtropical environment and **the thermal tolerance of triploids** in laboratory.

Identifying triploid advantages of abalone

02 Chemical induction of triploid abalone

Chemical induction of triploid abalone



- Study subjects： ***H. discus hannai*** **,DD and** ***H. discus hannai*** **♀ × *H. fulgens* ♂, DF**
- Method： **Chemical induction**
- Chemicals： **Cytochalasin-B (CB) and 6-dimethylaminopurine (6-DMAP)**
- Principles： **Inhibiting polar body II (PB2)**
- Inducing factors： **Four concentrations and three durations**
- Ploidy determination： **Flow cytometry， triploidy rates of 24 h PF larvae**
- Hatching index： **cleavage rate and hatching rate (24 PF)**

Experimental design for the autotriploid (*H. discus hannai*) and allotriploid (*H. discus hannai* ♀ × *H. fulgens* ♂) induction

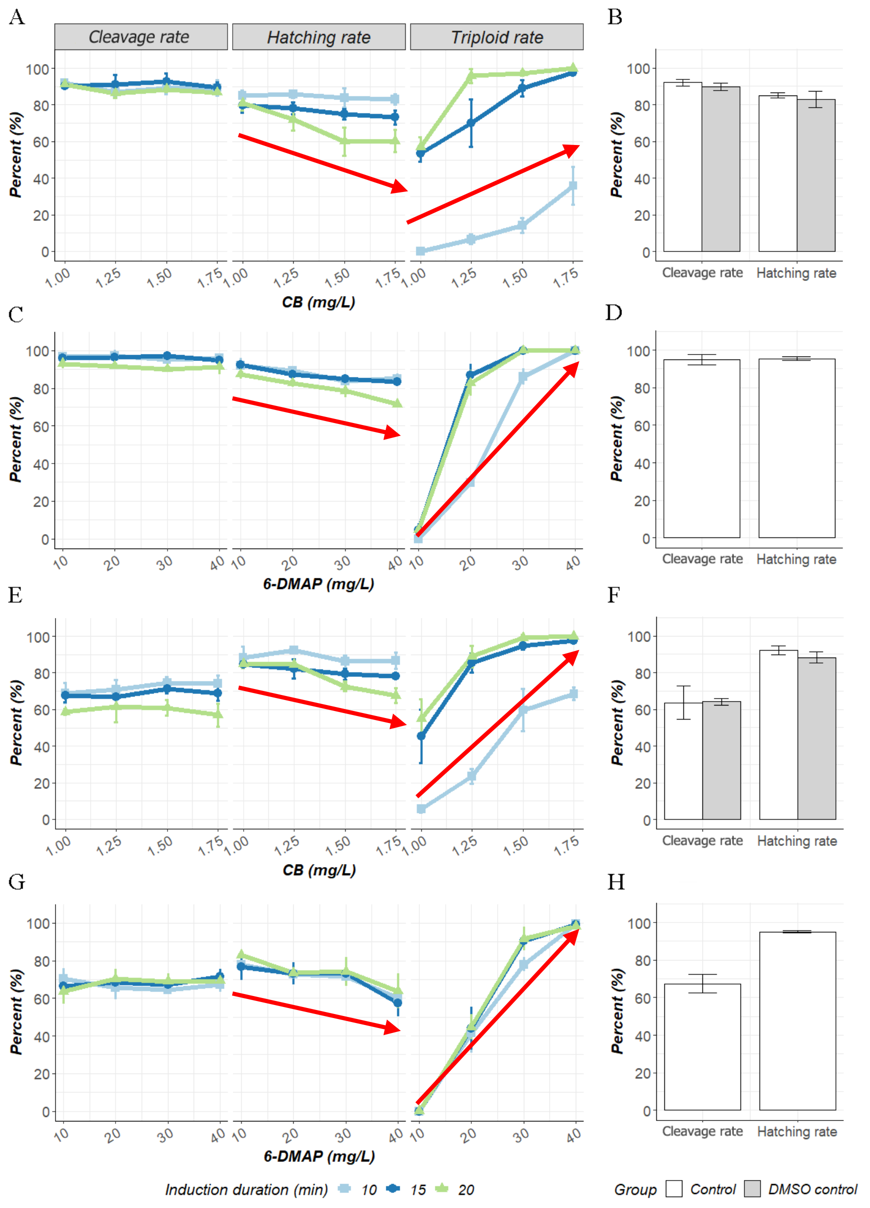
Items	Parents (F × M)	Treatments	Concentrations (mg L^{-1})	Starting time	Duration (min)
DDD-CB	10 DD × 5 DD	CB	1.00, 1.25, 1.50, 1.75	70% PB1	10, 15, 20
DDD-6-DMAP	10 DD × 5 DD	6-DMAP	10, 20, 30, 40	70% PB1	10, 15, 20
DDF-CB	10 DD × 5 FF	CB	1.00, 1.25, 1.50, 1.75	70% PB1	10, 15, 20
DDF-6-DMAP	10 DD × 5 FF	6-DMAP	10, 20, 30, 40	70% PB1	10, 15, 20

Chemical induction of triploid abalone

Summary of concentration, duration effects, and their interaction, on cleavage, hatching and triploidy of four treatments

Source	Dependent variable	*P*			
		DDD-CB	DDD-6-DMAP	DDF-CB	DDF-6-DMAP
Concentrations	Cleavage rate	0.124‡	0.664†	0.389‡	0.542‡
	Hatching rate	**< 0.001**‡	**< 0.001**‡	**0.018**†	**< 0.001**‡
	Triploid rate	**0.042**†	**< 0.001**†	**< 0.001**†	**< 0.001**†
Duration	Cleavage rate	0.121‡	< 0.001†	< 0.001‡	0.659‡
	Hatching rate	**< 0.001**‡	**< 0.001**‡	**0.001**†	0.274‡
	Triploid rate	**< 0.001**†	0.308†	**< 0.001**†	0.957†
Concentrations *Duration	Cleavage rate	0.573‡	0.591†	0.802‡	0.292‡
	Hatching rate	**0.014**‡	**0.018**‡	0.769†	0.940‡
	Triploid rate	0.990†	0.964†	0.985†	0.996†

- Higher triploid rate induced a low hatching rate and vice versa.
- The **hatching rate and triploid rate of autotriploid and allotriploid** were significantly affected by **CB and 6-DMAP concentration and CB duration**, while the 6-DMAP duration only affected the hatching rate of autotriploid larvae.



Chemical induction of triploid abalone

General evaluation index:

$$I_e = \frac{HR_e}{HR_c} \times TR_e$$

The relative proportion of triploid normal veligers.

General evaluation index (Ie) of autotriploid (*H. discus hannai*) by inhibiting PB2 with CB or 6-DMAP

CB	Induction duration (min)			6-DMAP	Induction duration (min)		
(mg L^{-1})	10	15	20	(mg L^{-1})	10	15	20
1.00	0.00 ± 0.00†	49.19 ± 4.07^{bc}	54.24 ± 4.97^{bc}	10	0.00 ± 0.00†	4.54 ± 3.09^{c}	4.21 ± 0.41^{c}
1.25	6.71 ± 2.84^{e}	**64.52 ± 12.06^{ab}**	**81.20 ± 9.04^{a}**	20	27.81 ± 1.18^{d}	79.29 ± 4.32^{ab}	71.67 ± 6.15^{ab}
1.50	14.10 ± 4.74^{de}	**78.54 ± 6.89^{a}**	**68.54 ± 8.22^{ab}**	30	**75.17 ± 3.39^{ab}**	**88.95 ± 2.25^{a}**	**82.23 ± 3.75^{ab}**
1.75	35.18 ± 11.09^{cd}	**83.96 ± 4.65^{a}**	**70.93 ± 7.23^{ab}**	40	**89.23 ± 2.56^{a}**	**87.47 ± 2.07^{a}**	**74.78 ± 2.08^{b}**

General evaluation index (Ie) of allotriploid (*H. discus hannai* ♀ × *H. fulgens* ♂) by inhibiting PB2 with CB or 6-DMAP

CB	Induction duration (min)			6-DMAP	Induction duration (min)		
(mg L^{-1})	10	15	20	(mg L^{-1})	10	15	20
1.00	5.35 ± 1.60^{f}	41.67 ± 13.38^{de}	50.84 ± 10.43^{cd}	10	0.00 ± 0.00†	0.00 ± 0.00†	0.00 ± 0.00†
1.25	23.62 ± 4.29^{ef}	**76.10 ± 7.18^{ab}**	**81.59 ± 5.30^{a}**	20	31.95 ± 9.89^{b}	33.81 ± 9.38^{b}	34.54 ± 5.02^{b}
1.50	55.69 ± 10.75^{bcd}	**81.38 ± 4.79^{a}**	**77.84 ± 2.94^{a}**	30	**58.71 ± 5.18^{a}**	**69.86 ± 5.30^{a}**	**71.72 ± 9.34^{a}**
1.75	64.36 ± 4.65^{abc}	**82.97 ± 1.06^{a}**	**73.22 ± 4.41^{ab}**	40	**63.15 ± 7.45^{a}**	**59.87 ± 7.73^{a}**	**65.79 ± 8.78^{a}**

- **The effective range**: CB 1.25 – 1.75 mg/L 15 – 20 min

  6-DMAP 30 – 40 mg/L 10 – 20 min

  → **Hatching rate: 57.25% – 85.20%**
  Triploid rate: 70.22% – 100.00%

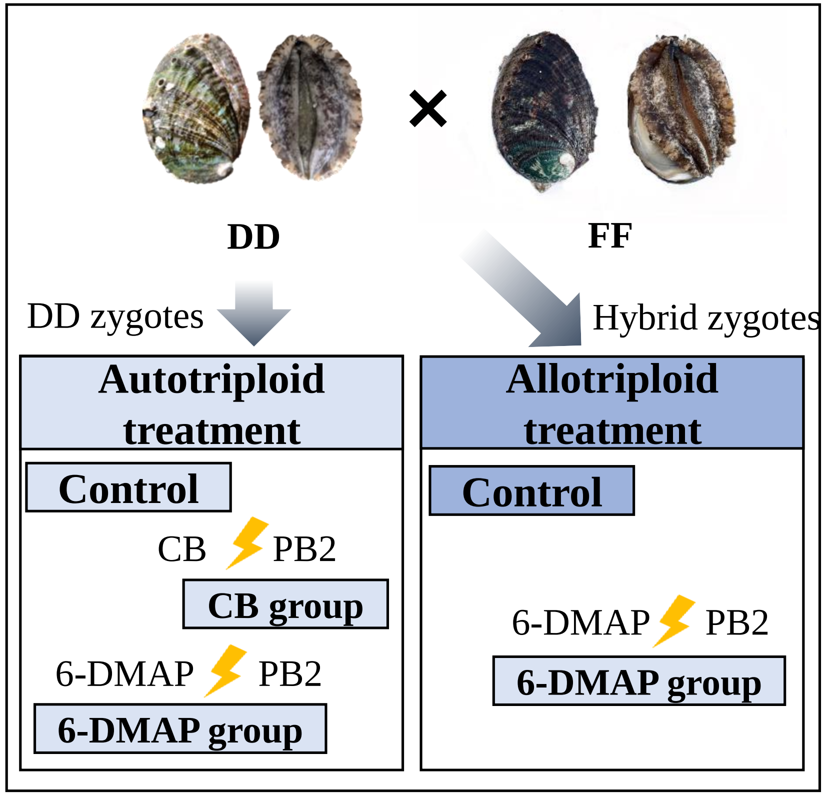
- **Optimal Induction condition:** Autotriploid: CB 1.75 mg/L 15 min, 6-DMAP 40 mg/L 10 min

  Allotriploid: CB 1.75 mg/L 15 min, 6-DMAP 30 mg/L 20 min

  → **Hatching rate: 73.11% – 85.20%**
  Triploid rate: 91.66% – 100.00%

03 Phenotypic traits assessment

Phenotypic traits assessment of autotriploid and allotriploid

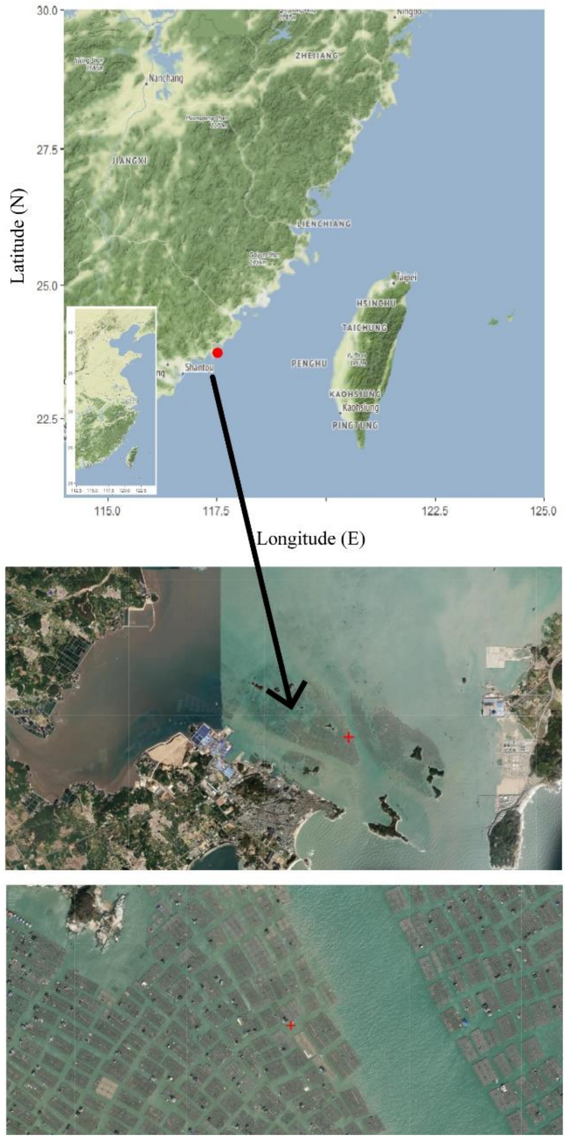


- Materials: **Autotriploid treatment**
  Allotriploid treatment
- Location: Zhangzhou, Fujian province, China
- Culture mode: **sea-based suspended systems**
- Initial placement time: **7 mpf**
- Initial shell lengths: 15.00 – 25.00 mm
- Period： **2019.04 – 2021.04** (**7 mpf – 30 mpf**)
- Phenotypic traits: **Triploid rate**, survival, **growth** (shell length, body weight, **meat yield**) and the **thermal tolerance**

Foot muscle weight 和

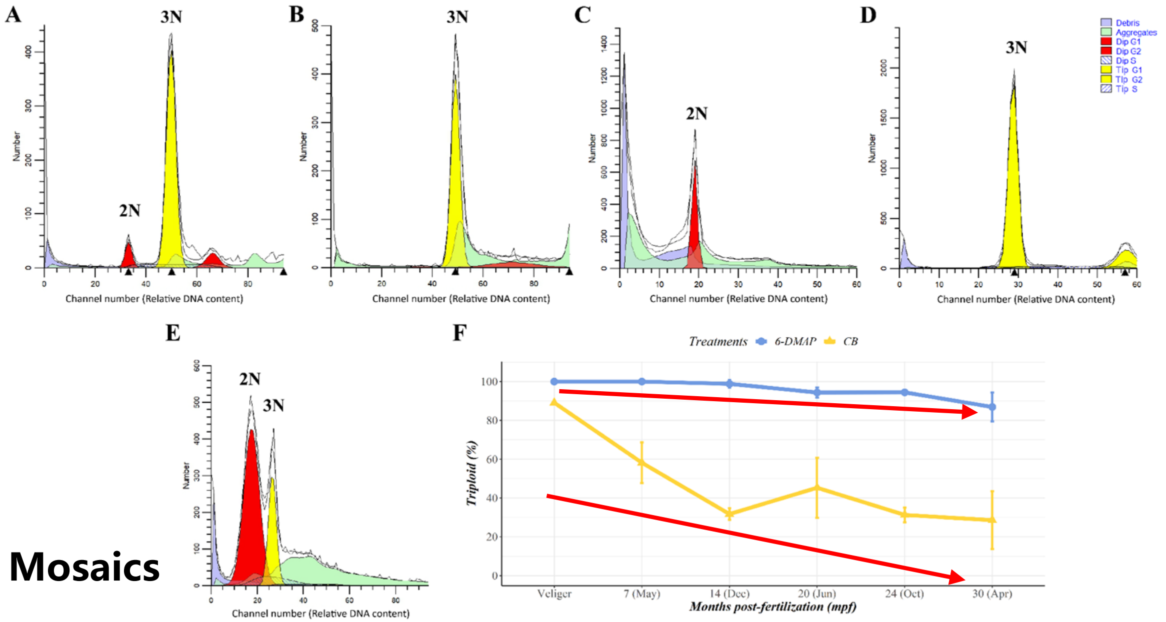
Foot muscle-soft tissue index：

$$FMSI = \frac{Foot\ muscle\ weight}{Body\ weight - shell\ weight} \times 100$$

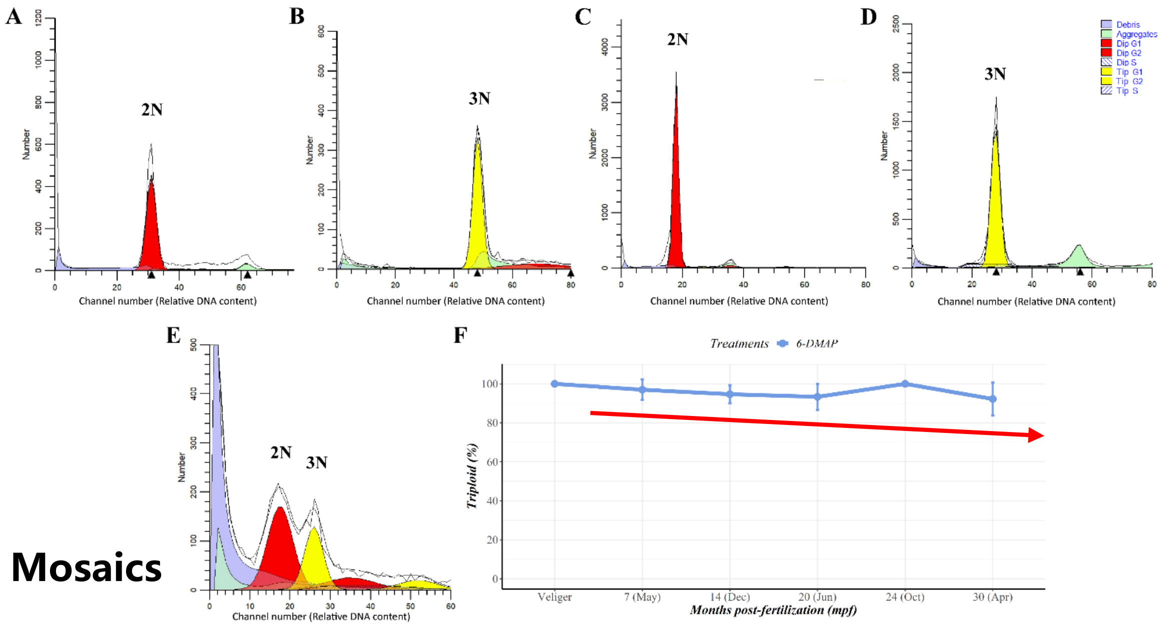


The sea-based suspended system

Triploid rate



Flow cytometry analysis and proportion of triploid larvae, juveniles, and adults of *H. discus hannai* obtained at different months post-fertilization (mpf) in two treated groups (CB and 6-DMAP)



Flow cytometry analysis and proportion of triploid larvae, juveniles, and adults of *H. discus hannai* ♀ × *Haliotis fulgens* ♂ obtained at different months post-fertilization (mpf) in two treated groups (CB and 6-DMAP)

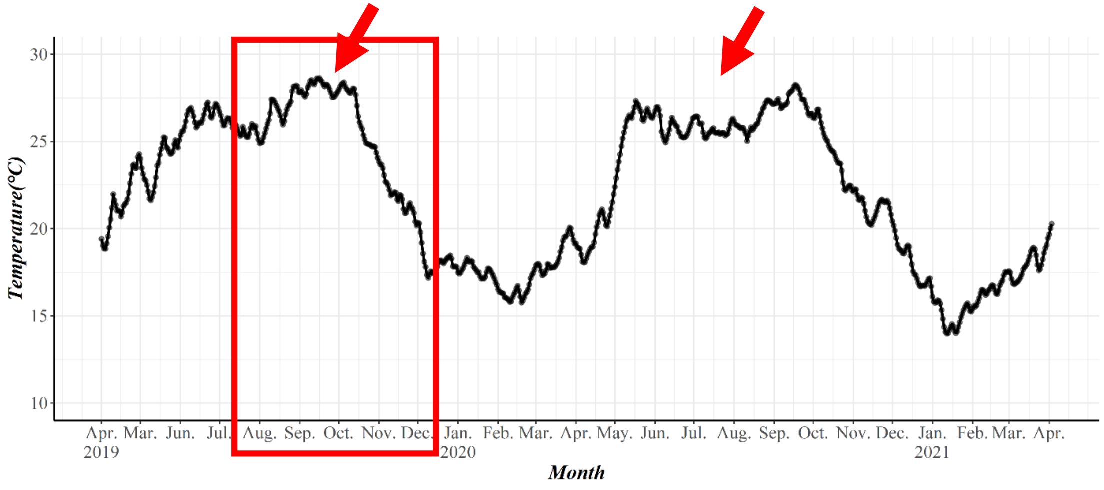
The ploidy status of autotriploid treatment:

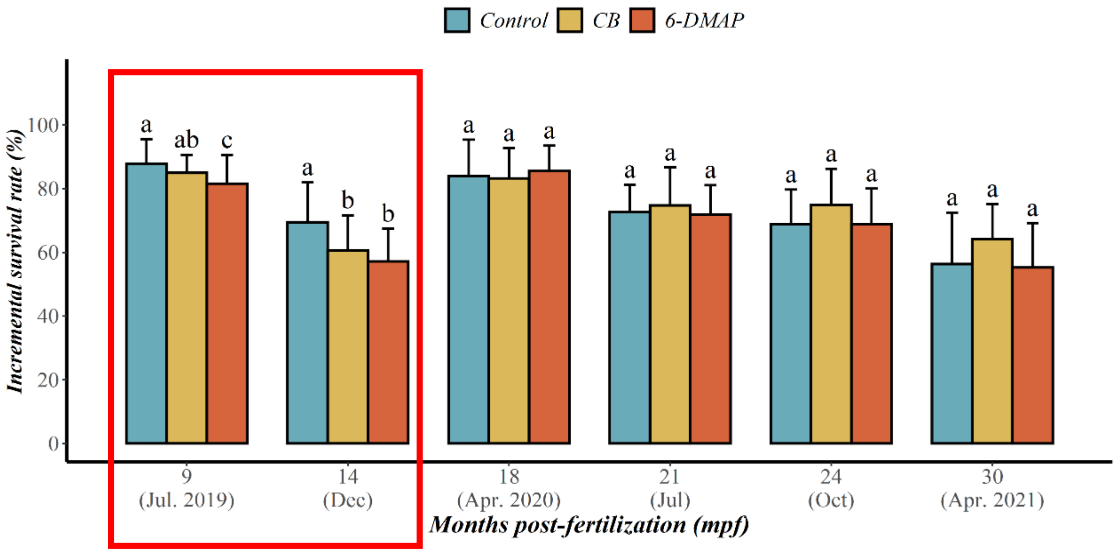
	Larval stage		30 months post-fertilization (mpf)
CB group	**89.10%**	→	**28.58%**
6-DMAP	**100.00%**	→	**86.91%**

The ploidy status of allotriploid treatment:

	Larval stage		30 mpf
6-DMAP group	**100.00%**	→	**92.22%**

Incremental survival rate of autotriploid treatment





Triploid advantage：

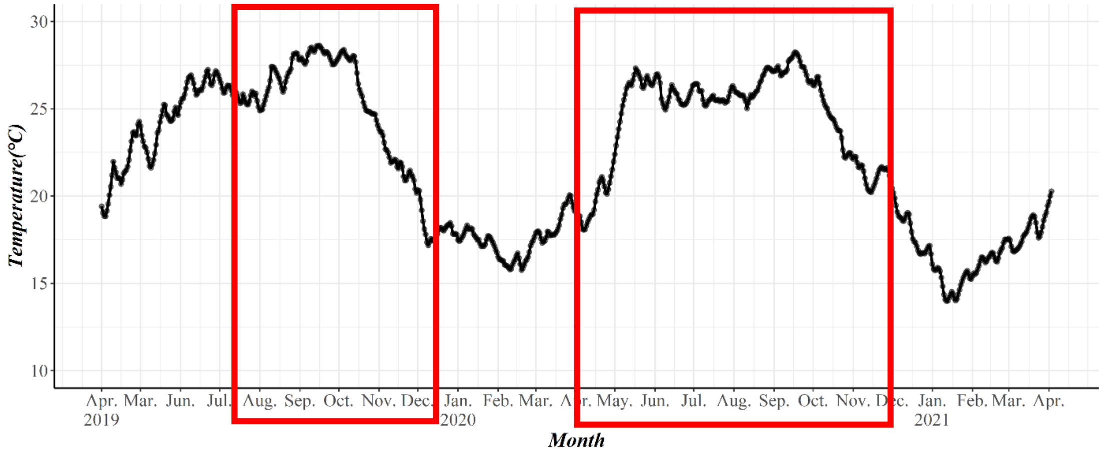
$$TA = \frac{P_{3N} - P_{2N}}{P_{2N}} \times 100$$

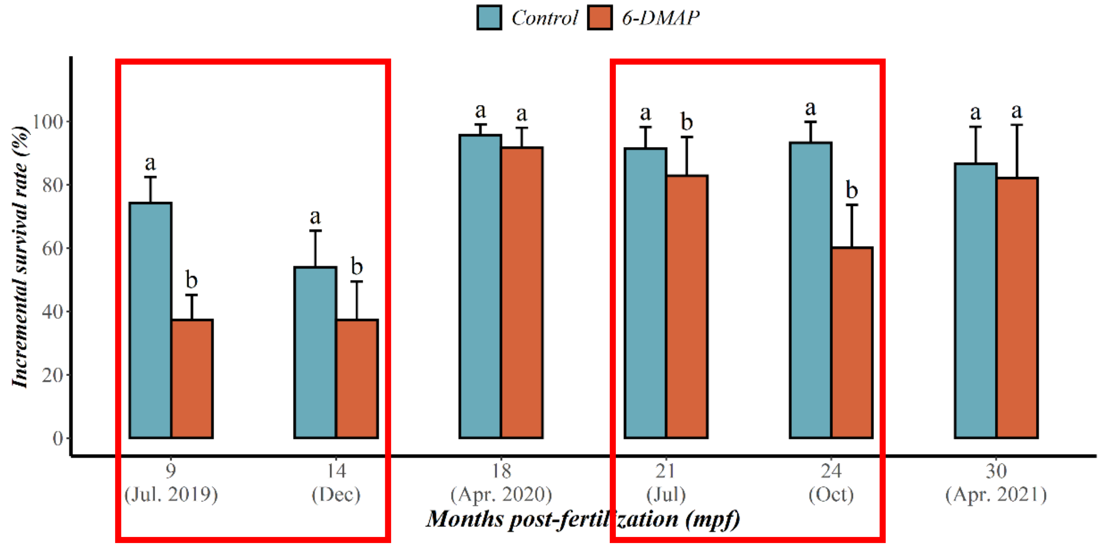
Triploid advantage (TA) for incremental survival rate in two groups (CB and 6-DMAP)

	Group	Months post-fertilization					
		9 (Jul)	14 (Dec)	18 (Apr)	21 (Jul)	24 (Oct)	30 (Apr)
TA (%)	CB	−3.17	**−12.72**	−0.84	2.90	8.76	13.88
	6-DMAP	**−7.05**	**−17.71**	1.89	−1.10	0.05	−1.90

- Water temperature: 13.98 – 28.63 ℃ (2019.04 – 2021.04); **High-temperature periods**: **23.48 – 28.63 °C** (mid-May to mid-October) .
- Incremental survival rate: **Significant decreases** of CB and 6-DMAP group **during the first high-temperature period.**

Incremental survival rate of allotriploid treatment





Triploid advantage：

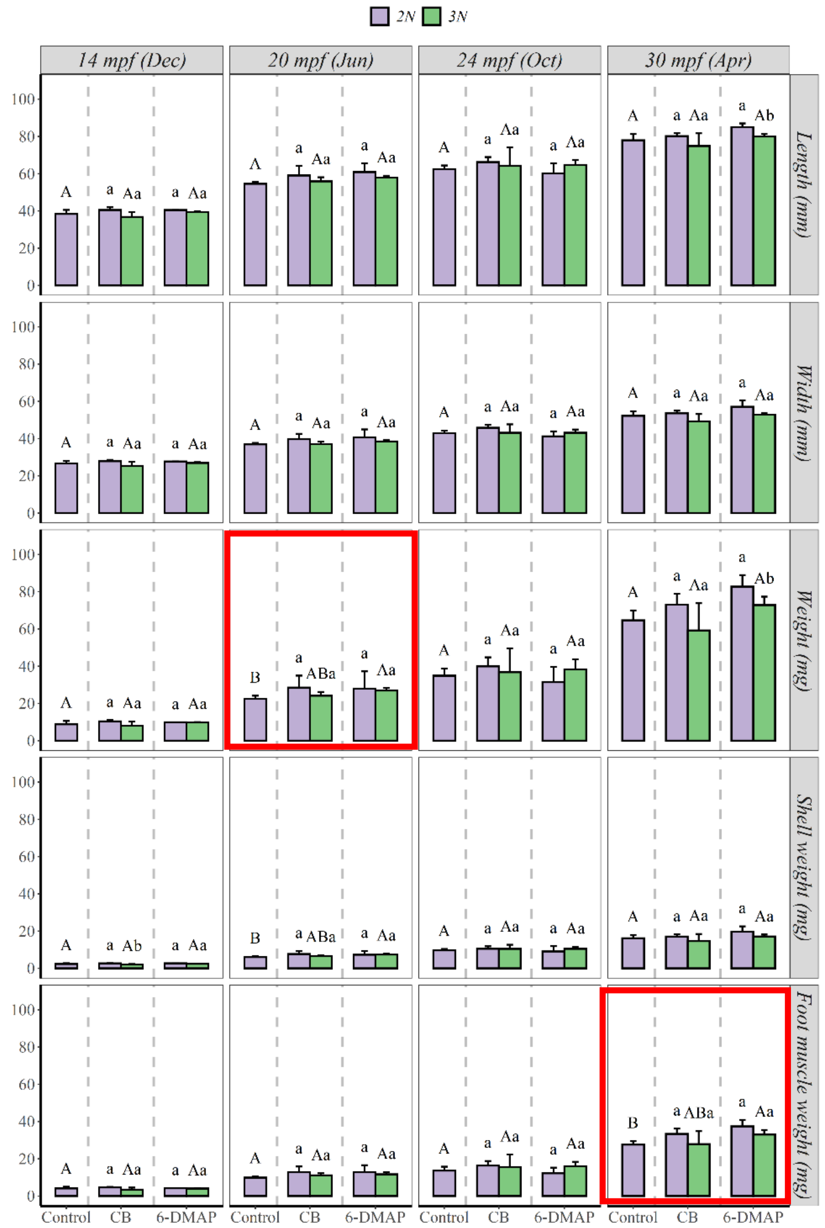
$$TA = \frac{P_{3N} - P_{2N}}{P_{2N}} \times 100$$

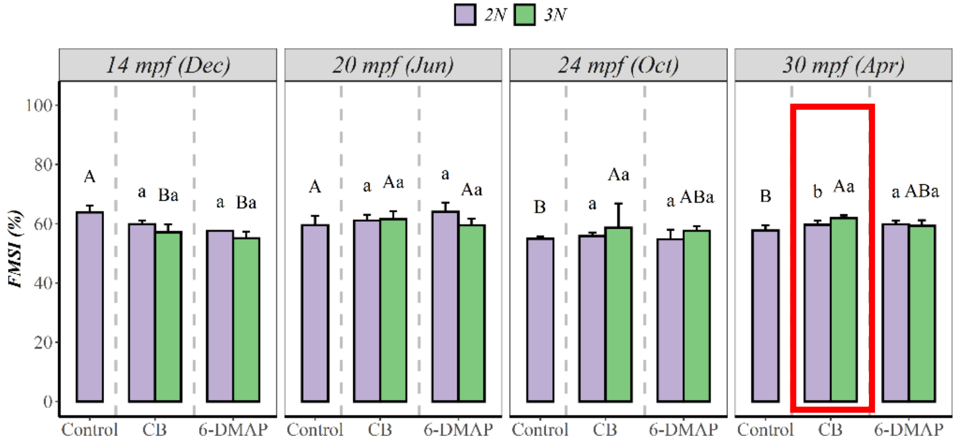
Triploid advantage (TA) for incremental survival rate in 6-DMAP group

	Group	Months post-fertilization					
		9 (Jul)	14 (Dec)	18 (Apr)	21 (Jul)	24 (Oct)	30 (Apr)
TA (%)	6-DMAP	**−49.75**	**−30.81**	−4.01	**-9.35**	**−35.61**	-5.13

- **Significant decreases** of Incremental survival rate in 6-DMAP group **during the initial stage and the high-temperature period** (Jul – Dec and Apr – Dec).

Growth and meat yield of autotriploid treatment



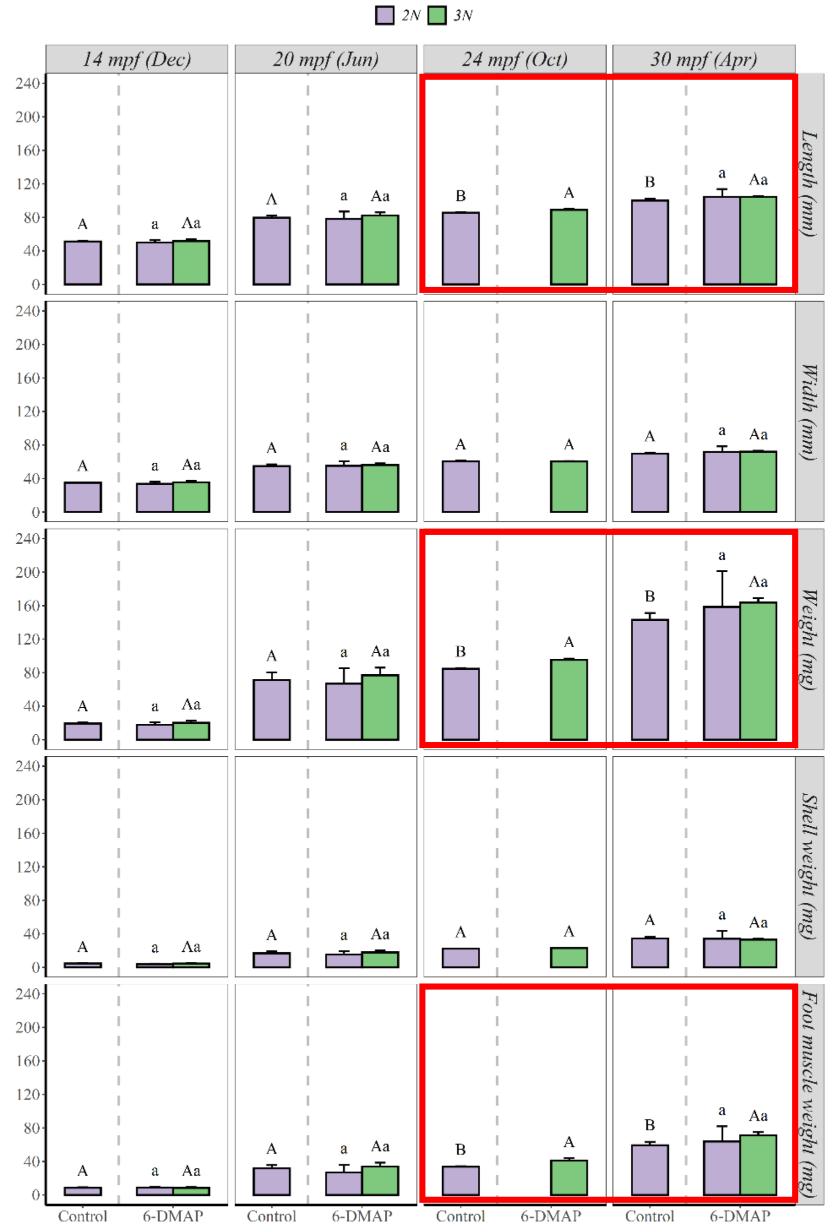


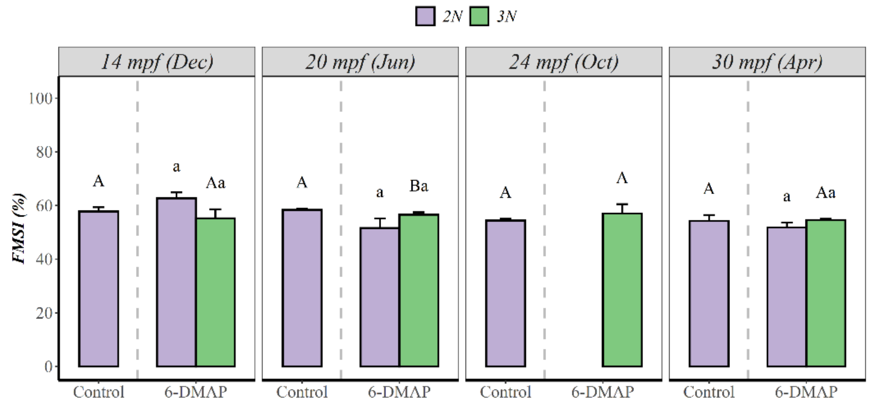
Triploid advantage (TA) for productive traits in triploid (3 N) within two treated groups and control diploid

Productive traits	14 mpf (Dec)		20 mpf (Jun)		24 mpf (Oct)		30 mpf (Apr)	
	CB 3N	6-DMAP 3N	CB 3N	6-DMAP 3N	CB 3N	6-DMAP 3N	CB 3N	6-DMAP 3N
Shell length	−4.31	2.51	2.40	6.70	2.88	3.60	−3.90	2.62
Shell width	−5.23	1.11	0.50	4.23	0.41	0.45	−5.70	1.19
Body weight	−9.03	10.96	7.52	**19.80**	5.50	9.79	−8.46	12.64
Shell weight	−13.59	2.89	**8.91**	**22.84**	7.75	7.86	−9.29	5.75
Foot muscle weight	−16.23	−1.52	12.66	18.30	13.77	17.45	0.85	**19.57**
FWSI	−10.48	−13.65	3.35	−0.12	**6.75**	4.89	**7.22**	2.70

- **Triploids in the 6-DMAP group** showed superior growth in **body weight** at 20 mpf. TA = 19.80%。
- **After the end of the maturation season (30 mpf), significant increase in foot muscle weight (19.57%) and FMSI (7.22%)** were found **in the 6-DMAP and CB group triploids.**

Growth and meat yield of allotriploid treatment





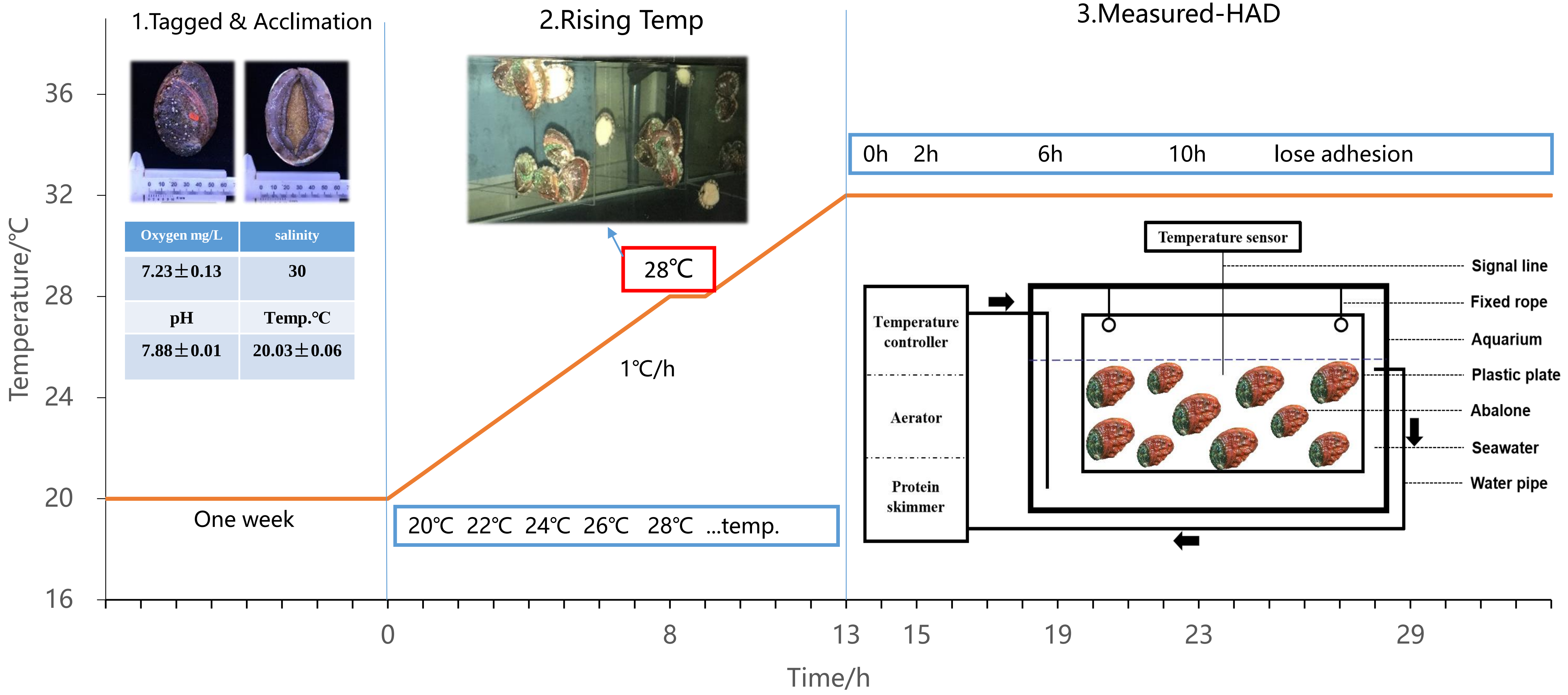
Triploid advantage (TA) for productive traits in triploid (3 N) within 6-DMAP group and control diploid

Productive traits	Months post-fertilization			
	14 mpf (Dec)	20 mpf (Jun)	24 mpf (Oct)	30 mpf (Apr)
Shell length	0.84	3.29	**3.97**	**4.40**
Shell width	1.40	2.33	−0.08	3.29
Body weight	4.27	8.01	**12.55**	**14.32**
Shell weight	-1.69	6.02	3.42	−3.94
Foot muscle weight	-1.98	6.99	**22.16**	**19.80**
FWSI	-4.51	**-2.99**	4.85	0.59

- **The shell length (4.40%), body weight (14.32%) and foot muscle weight (19.80%) of the 6-DMAP group allotriploids were significant increase** during the maturation season (24 mpf) and after the season (30 mpf).

Thermal tolerance of autotriploid

- **Heat stress attachment duration (HAD)** was applied to assess **the thermal tolerance of diploids and triploids**, which were derived from the control and 6-DMAP groups in **autotriploid treatment**, respectively.



Thermal tolerance of autotriploid

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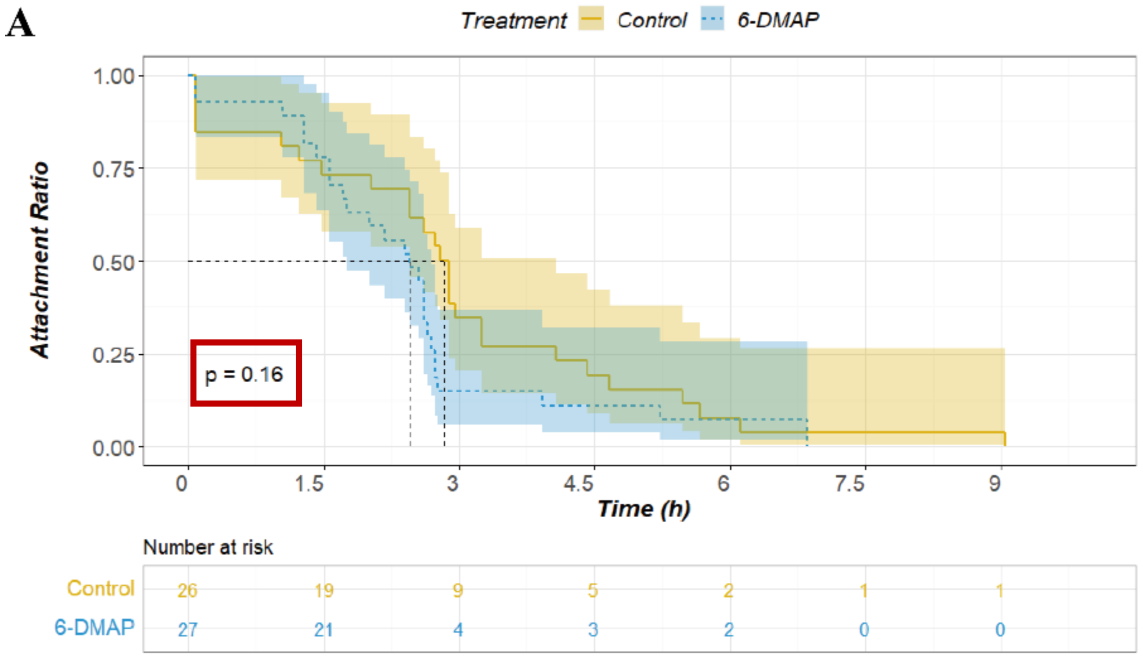
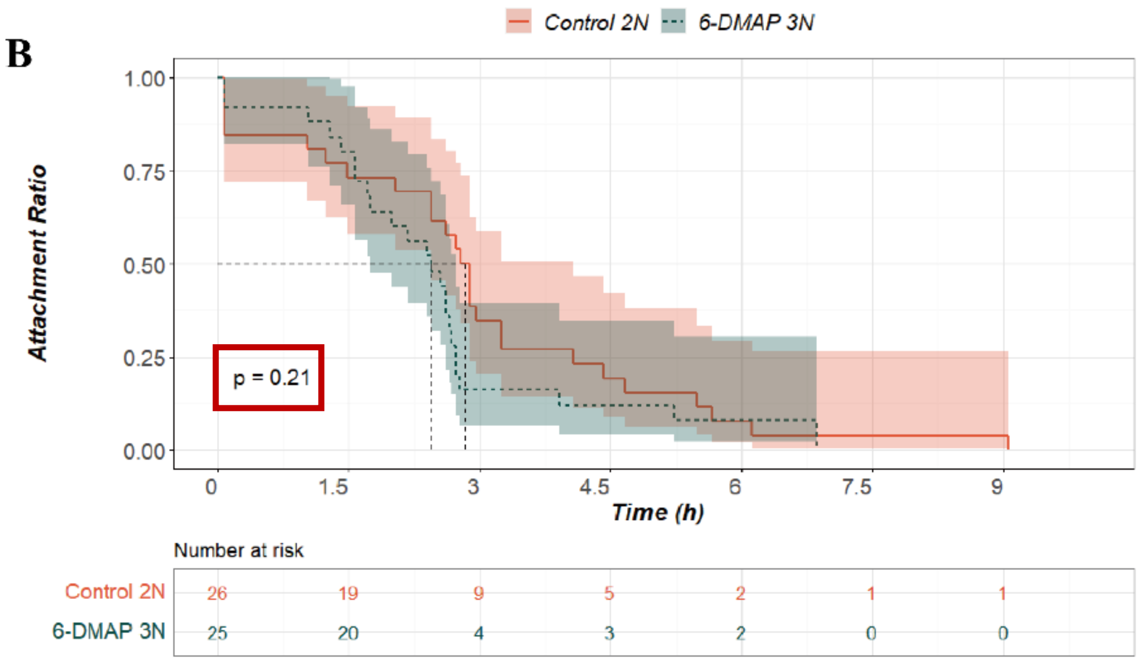
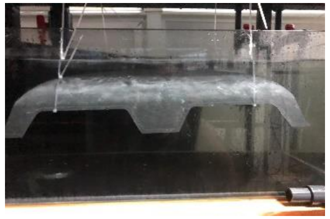


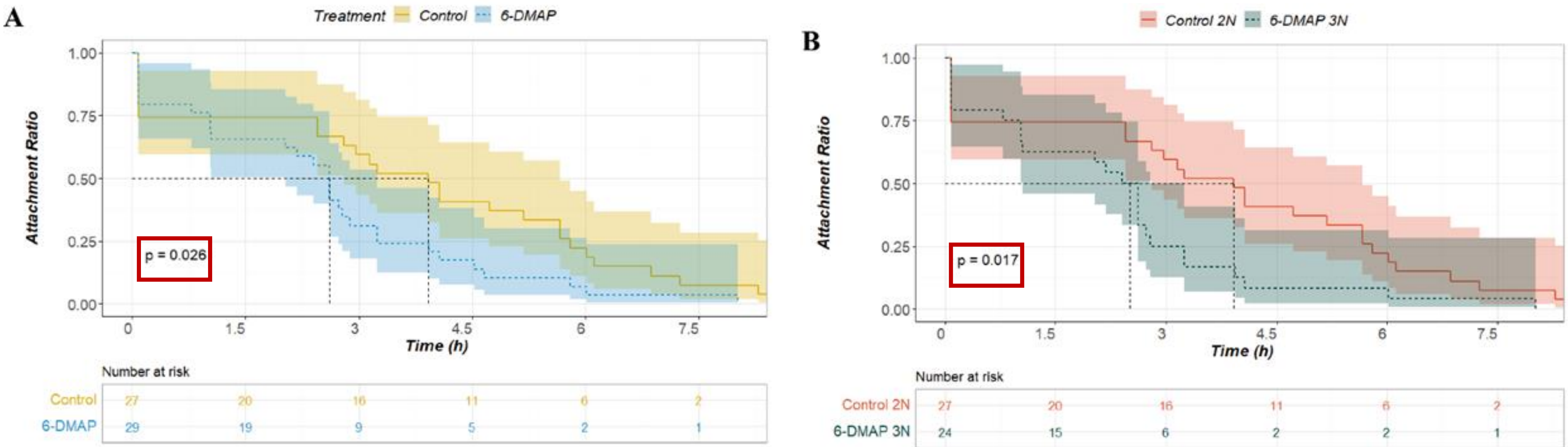


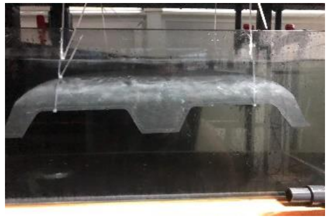


- **Autoploid: No difference in thermal tolerance between autotriploids and diploids.**

Thermal tolerance of allotriploid

- **Heat stress attachment duration (HAD)** was applied to assess **the thermal tolerance of diploids and triploids**, which were derived from the control and 6-DMAP groups in **allotriploid treatment**, respectively.





- **Allotriploid: The thermal tolerance of allotriploid decreased significantly.**

04 Conclusion

Conclusion

01.

Highly efficient procedure for inducing autotriploid (*H. discus hannai*) and allotriploid (*H. discus hannai* ♀ × *H. fulgens* ♂) using chemical method was established. A relatively high rate of triploid individuals was obtained, ranging from 91.66% to 100.00% using optimal parameters.

02.

The disadvantage of survival has been found in autotriploid and allotriploid.

03.

Autotriploid and allotriploid showed the advantages of growth and meat yield. It may be attributed to the hypothesis of the diversion of energy from gonadal to somatic growth.

04.

There was no difference in thermal tolerance between autotriploids and diploids but the thermal tolerance of allotriploid decreased significantly.

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Thank you！