Trials on New Methods for Seed Culture in Japanese Abalones

Yasuyuki Koike, A.E. Stott, F. Ahmed, T. Takeuchi, C. Strussman, M. Yokota, S. Segawa, and S. Watanabe

Abstract Abalones are one of the most important coastal animal resources in Japan, and the study of their seed culture has a long history of more than 40 years. Most of the produced seeds have been released to the seabeds. The total number released nowadays is almost 3 million (small individuals) per year. However, the annual production of natural abalones has decreased remarkably in the last 10 years.

Still, the culturing of market-size abalones has been started in several regions in Japan, such as *Haliotis discus* in the south and *H. discus hannai* in the north. The production of cultured abalones is slowly increasing. This tendency has the benefits of preventing over catching of natural stocks and reducing the importation of cultured abalone from foreign countries.

Under these conditions, we tried to improve this trend with some experiments on seed culture methods.

1. Using artificial food (a microparticle diet) to replace natural diatoms for feeding post-larval abalones to improve the survival and growth rates
2. Trials for the improvement of artificial production of inter-specific hybrids among three large size species

The result will lead to recommendations for newly developed adult cultures in closed spaces. The possibility of producing regional specialities is suggested.

Y. Koike (✉), A.E. Stott, F. Ahmed, T. Takeuchi, C. Strussman, M. Yokota, S. Segawa, and S. Watanabe
Department of Ocean Science, Tokyo University of Marine Science and Technology, 4–5–7 Konan, Minato-ku, Tokyo, 108–8477, Japan
e-mail: oreillemer@ybb.ne.jp

1 Introduction

Abalones are one of the most important coastal animal resources in Japan, and the study of their seed culture has a long history of more than 40 years. Most of the produced seeds have been released to the natural seabed for restocking. Currently, the total number of released seed abalones is almost 3 million per year. However, the annual production from natural stocks has decreased remarkably in the last 10 years.

The culturing of market size abalone has been started in several regions, such as *Haliotis discus discus* in the south of Japan, for example, in the Matsuyama region in Ehime Prefecture, and *H. discus hannai* in the north, for example, in Hokkaido and in Kesennuma in Miyagi Prefecture. The production of cultured abalone is increasing progressively. This tendency has the benefits of preventing over catching of natural stocks and reducing the importation of cultured abalone from foreign countries.

Under these conditions, we tried to improve this trend with some experiments on seed culture methods as follows. The first experiment involved replacing natural food with microparticle artificial food to feed post-larval abalones. A new post-larval abalone culture system, Stott's abalone post-larval production system (SAPPS), was tested using commercially available artificial diets. The second experiment involved improving hybrids among the three principal species. These experiments were conducted from 2000 to 2005 at Tateyama Station-Banda, Tokyo University of Marine Science and Technology (former Banda Marine Laboratory, Tokyo University of Fisheries).

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2 First Experiment

We used artificial food (microparticle diet) to replace natural food (diatoms) for feeding post-larval abalones to improve the survival and growth rates.

2.1 Materials and Methods

The experiment was conducted over a period of 4 weeks to test the SAPPS method (Fig. 1) against the Diatom method (Table 1). Two plates from each treatment of SAPPS-Cos (diet supplied by Cosmo, Matsuyama, Japan), SAPPS-Adam (diet supplied by Adam and Amos, Mt Barker, SA, Australia), and Diatom were placed into 10-l flow-through tanks (three treatments replicated three times each). Brood stock at Banda Marine Laboratory was induced to spawn using UV light, and the resulting larvae were used in the trial when they were deemed ready to settle. Once the larvae had metamorphosed into post-larvae (on the experimental plates), the trial was commenced to compare SAPPS (using the two commercial diets) to Diatom in terms of post-larval growth and survival. Food was resprayed onto plates every second day, and measurement for post-larval survival was calculated every second day.

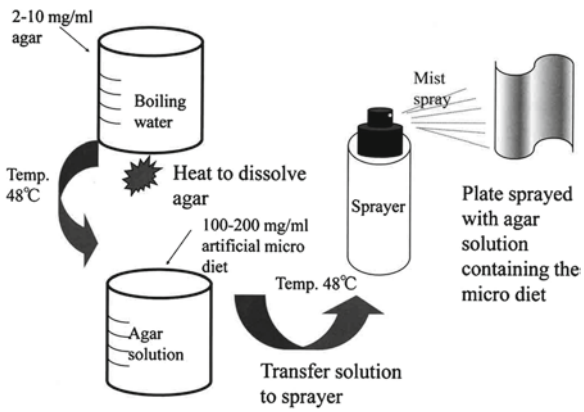

Fig. 1 Stott's abalone post-larval production system (SAPPS)

Table 1 Main species and density of diatoms that were present on the collector plates of spats

Species	Cell density (%)	Final density (cells/cl)
Navicula spp.	40	8.4×10^4
Nitzschia spp.	3	6.3×10^3
Amphora bigibba	12	2.3×10^4
Entomoneis sp.	3	6.3×10^3
Melosira nummuloides	2	4.2×10^3
Diploneis sp.	1	2.1×10^3
Tabularia sp.	2	4.2×10^3
Amphora spp.	4	8.4×10^3
Cyclophora tenuis	3	6.3×10^3
Others	30	6.3×10^4
Total	100	21 ± 0.3 (1×10^6) (Scott et al. 2004)

2.2 Results and Conclusion of the First Experiment

The Cos diet, when compared to the Adam diet, had higher moisture, lipid and ash contents, and lower protein content (Table 2). Final survival of post-larvae was significantly higher in SAPPS-Cos (56.7 ± 11.15%) compared to the other three treatments [9.4 ± 2.7%, 8.5 ± 1.1% and 3.0 ± 2.4% for the Diatom, SAPPS-Adam and PPS-Adam diets, respectively. The final length of post-larvae in SAPPS-Cos (1,065 ± 73 μm) was significantly higher than those in the other treatments (average of 812–883 μm) (Table 3, Fig. 2).

The Cosmo diet was superior to the Adam diet in terms of growth and survival of post-larval abalone. The level of protein in the Adam diet could have been too high, and the level of lipid too low. SAPPS was

Table 2 The size and composition of the different diets

	Diet *		
	Adam *	Cosmo *	Diatom
Particle size – wet (μm)	43 ± 16	38 ± 13	–
– dry (μm)	32 ± 10	28 ± 8	–
Moisture (%)	7.5	6.8	1.6
	On dry matter basis (%)		
Crude protein	54.4	34.3	18.3
Crude lipid	2.5	4.9	8.1
Crude ash	14.1	17.1	52.8 (Scott et al. 2004)

*Diet Adam: supplied by Adam and Amos; Mt. Barker, SA, Australia

Diet Cosmo: supplied by Cosmo; Matsuyama, Japan

Table 3 Results in the three different treatments for *Haliotis discus discus*

Treatment	No. larvae settled	Final Shell length (μm)	Daily growth rate (μm)	Survival rate (%)
SAPPS – Adam	377	841	19	8.5
PPS – Adam	243	812	18	3.0
SAPPS – Cos	334	1,065	27	56.7
Diatom	381	883	21	9.4
				(Scott et al. 2004)

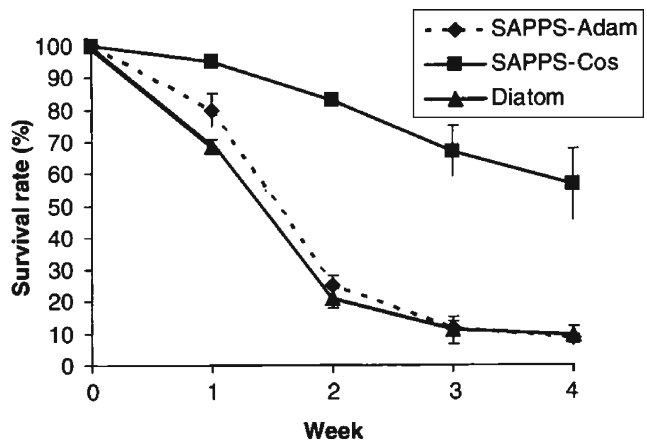

Fig. 2 The average percentage survival (± standard deviation) of postlarval *Haliotis discus* in the three different treatments (trial 1)

superior in terms of growth and survival of post-larvae when compared to the other two methods. This is possibly because SAPPS is a continuous system where artificial food is resprayed onto wet plates that have living post-larvae attached. There is potential for SAPPS to be used instead of, or together with, the current diatom method in the production of post-larval abalone.

3 Second Experiment

Trials were conducted to improve the artificial production of interspecific hybrids among three principal species.

3.1 *Materials and Methods*

Adult *Haliotis discus discus* (HDD), *H. gigantea* (HG) and *H. madaka* (HM) were collected from Tateyama Bay, in the offing of Tateyama Station-Banda. Crossbreeding between these principal species was attempted in several trials from 1985 to 2002. Spawning was induced with ultraviolet-irradiated seawater and heat shock, common methods used in the abalone hatcheries in Japan. The fertilization rates of homologous crosses and the hybrids were estimated. The produced hybrids were reared in separate tanks, avoiding mixing with the other hybrids or pure strains.

For the reproductive viability of the hybrids, HM×HDD and HDD×HM hybrids were induced to spawn. The fertilization rate and number of hatched trochophore larvae were estimated, and development of larvae was monitored to observe progeny viability of F2HMxHDD and HDDxHM hybrids. For the gonad characteristics of the hybrids, gonads of at least 3-year-old abalones were collected from September 2005 to January 2006. The fixed gonads were sectioned at a thickness of 8 μm and stained with Mayer's hematoxylin and eosin. Slides were observed to establish sex, the most abundant gametogenic cell type present and the most advanced gametic cell

type present in each abalone. The gametic cell types and maturity stages for male and female abalones were identified following the method of Tomita (1967/68).

3.2 Results and Conclusions

All hybrids responded positively to spawning stimulation [HM×HDD (two males, one female); HDD×HM (four males, three females); HDD×HG (three males, two females) spawned] (Fig. 3). Fertilization rates of homologous gametes of *H. discus discus* and *H. madaka* were on average more than 80%, but they were significantly lower in *H. gigantea*. On the contrary, fertilization rates of heterologous gametes were less than 20% except the reciprocal crosses of *H. discus discus* and *H. madaka* (22.4% in DDxM, 60.8% in MxDD) (Table 4). For the F2 generation, the fertilization rate was averaged at 56% (40.9–68.5%).

In the back-crossing, fertilization rates averaged 45.6% (HDD-HDD×HG), 70.6% (HDD×HG-HDD) and 3% (HG-HDD×HG). The larvae followed the same development stages as described for abalones. At the end of 5 months of rearing, the F2 hybrids were, on average, 9.9 mm (4.94–13.58) in shell length and 0.14 g (0.04–0.3) g in body weight.

Table 4 The fertilization rate of homospecific, heterospecific, hybrid and back-cross (Faruq 2007; Koike et al. 1988)

Parents		No. of	Fertilization rate (%)	
Female	Male	trials	Average	Range
Homospecific crosses				
DD	DD	6	90	71.4–100
M	M	5	81	19–96.2
G	G	7	34	0.3–86.7
Heterospecific crosses				
DD	M	3	22	6–65.3
M	DD	3	61	3.4–97.6
G	M	3	2	0.4–8.5
M	G	3	5	1–9.6
G	DD	5	20	0.8–53.6
DD	G	4	1	0.5–4.2
Hybrid crosses				
DD×M	DD×M	1	59	40.2–87
M×DD	M×DD	1	69	58.9–76.3
DD×G	DD×G	1	41	38.5–45.1
Back-crosses				
DD	DD×G	1	46	42–53.3
DD×G	DD	1	71	64.7–74.2
G	DD×G	1	3	0.9–5.6

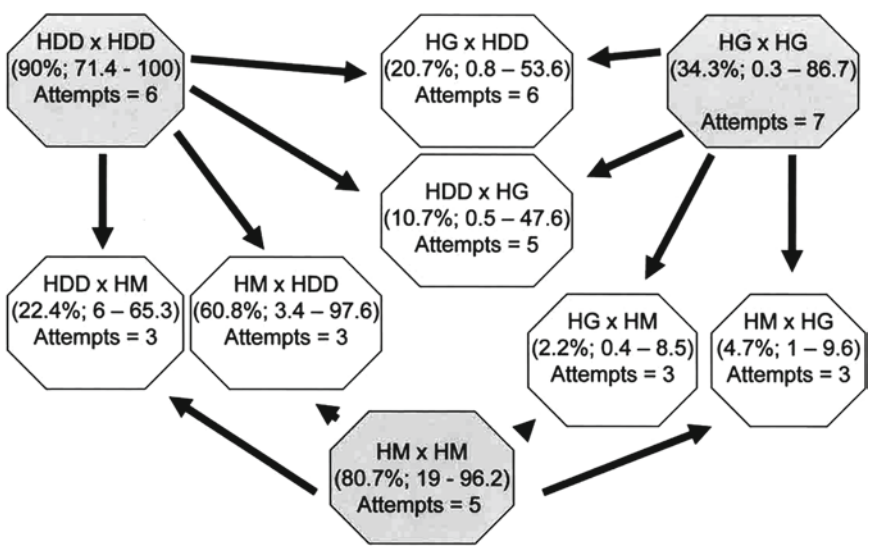

Fig. 3 Gamete combination (Female × Male) and fertilization rate [mean (%); range] of each combination (HDD = *H. discus discus*, HM = *H.madaka*, HG = *H. gigantea*) (Faruq 2007)

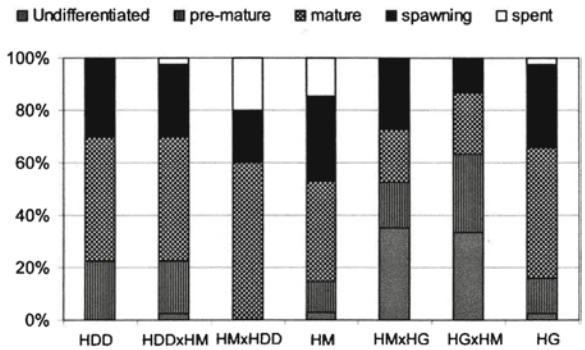

Fig. 4 Maturity stages in the three abalone species and their hybrids (HDD = *H. discus discus*; HM = *H. madaka*; HG = *H. gigantea*) (Faruq 2007)

Most hybrid abalones sampled for gonad analysis could be sexed. Comparison of gonad histology of the parental species with the hybrid suggested that the hybrids had similar gonad development as the parental species (Fig. 4). Hybrids in spawning stage were observed in the spawning season of their parental species from December to January.

It was observed that both male and female hybrids were able to produce and spawn gametes when they were stimulated by the conventional procedures. All four hybrid crosses for which histological observation of gonads was conducted were found to be proceeding through gametogenesis.

4 Conclusion

In conclusion, hybridization among principal abalones *H. discus discu*s, *H. gigante*a and *H. madaka* is possible, with crosses between *H. madak*a and *H. discus discus* being relatively easier to accomplish than the other four crosses. The former experiment proved that *H. discus discus* female × *H. madaka* male hybrids had growth performance similar to and in some cases higher than the parental species (Faruq 2007). This can be useful in attempts to produce back-crossed and F2 hybrids to obtain fast-growing strains.

To identify the hybridization and parenthood of the hybrid, analyses of allozymes and mtDNA are effective, and these aspects will be examined in future studies.

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