Single and mixed cultivation methods of transplanted *Pocillopora verrucosa* and *Stylophora pistillata* (Anthozoa) in Serangan planting areas, Bali, Indonesia

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Abstract. Damaged coral reefs with low cover can be repaired through various methods, one of which is transplantation. The objective of this research was to determine a method of culturing for *Stylophora pistillata* and *Pocillopora verrucosa* in the Serangan coast of Bali. Fragments of *S. pistillata* and *P. verrucosa* were transplanted into a substrate made from a mixture of cement and sand, after which they were cultured either in single species, or as a mix of both species. Data were collected weekly, from October 2016 to January 2017. The observed data consists of vertical growth, colony diameter, number of new polyps and environmental parameters. Growth performance was evaluated by t-test with a confidence level of <5%. The results showed that during the 12 weeks of cultivation, *S. pistillata* significantly presented faster vertical growth, a wider colony diameter and a higher number of polyps than *P. verrucosa*. Furthermore, coral transplantation in single species had a significantly better growth performance than the mixed cultivation. The coral transplantation of *S. pistillata* and *P. verrucosa* at the Serangan Beach was successful because the survival rate reaches 100%.
Key Words: coral, Denpasar, growth, transplantation.

Introduction. Coral reefs are underwater structures made from calcium carbonate secreted by corals, and most coral reefs are built from stony corals. Coral reefs are fragile ecosystems, in part because they are very sensitive to water temperatures. The coral reef ecosystem is composed of reefs from the class Anthozoa and the order Scleractinia (Tomascik et al 1997). It is estimated that more than 3000 species of marine biota can be found in coral reef ecosystems. It is estimated that as many as 590 species belonging to 80 genera of coral can be found in Indonesia (Suharsono 2008). However, currently, coral reefs face enormous threats from the impact of human activities, such as non-environmentally sound development, overfishing and pollution. They face numerous threats from climate change, oceanic acidification, blast fishing, cyanide fishing for aquarium fish, overuse of reef resources. Moreover, there are some harmful land-use practices, including urban and agricultural runoff and water pollution, which can harm reefs by encouraging excess algal growth (Supriharyono 2009; Ammar et al 2013).

Although coral reefs occupy only 0.1% of the ocean's surface, they are the world's richest repository of marine biodiversity, and coral reefs have survived over the course of more than 400 million years of evolution, possessing richness, diversity of life, and structures that are integral foundations for humanity (Okudo et al 2005; Ammar 2011). The importance of corals and coral reefs includes the following:

(i) corals remove and recycle carbon dioxide; the excessive amount of this gas contributes to global warming;

(ii) reefs buffer shorelines against harsh ocean storms and floods;

(iii) reefs provide resources for fisheries; food items include fish, crustaceans, and mollusks;

(iv) coral reefs attract millions of tourists every year;

(v) coral reefs are an intricate ecosystem and contain a diverse collection of organisms; without the reefs, the aforementioned organisms would die;

(vi) some evidence suggests that coral reefs can potentially provide important medicines, including anti-cancer drugs and a compound that blocks ultraviolet rays;

(vii) coral skeletons are being used as bone substitutes for reconstructive bone surgeries (Suharsono 2008; Ammar 2011).

Coral reefs suffered a sharp decline due to several reasons that are both natural and anthropogenic in nature. Efforts to improve coral reefs can be made by various methods, one of which is the transplant method. Research on coral transplantation methods has been performed by some researchers in Indonesia, such as Yunus et al (2013), using the hanging rope of fragments and the attachment of corals on the substrate. Other researchers (Prameliasari et al 2012) used the coral fragment size method on the hanging rope. Coral transplantation using hanging ropes with medium coral fragments yields the best coral growth. Therefore, it is very important to make improvement strategies to increase diversity and coral reefs cover.

In coral reef ecosystems, there is commensalism or competition relationships between species, so the species can either mutually benefit, or harm each other (Manaputty 1991). This study aimed to determine the differences in coral growth of *S. pistillata* and *P. verrucosa* transplants in single species and as a combination of species.

Material and Method

Description of the study sites. The study was conducted from October 2016 to January 2017, in Serangan District of Denpasar, Bali (Figure 1). Coastal Serangan waters are located in the southern Bali Island, about 5 km from Denpasar city. The research station was located approximately ±200 m on the beach from the coral reef flat, at a depth of about ±1 m during the lowest tide.

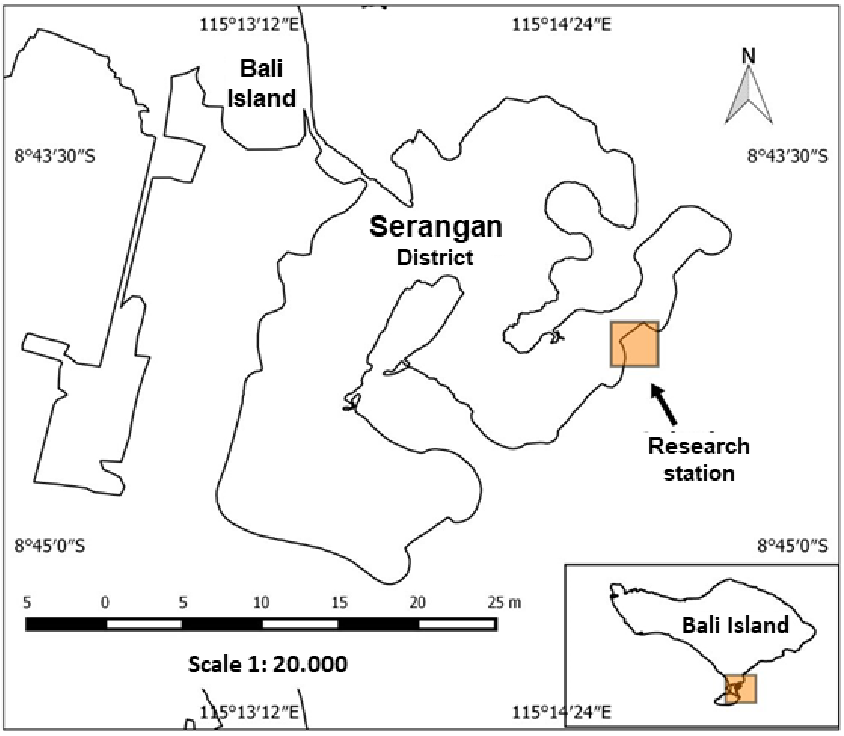

Figure 1. The research station at Serangan Island, Bali, Indonesia.

Procedures. The research was performed in four steps: preparation, implementation, observation and handling. The preparatory stage consisted in the preparation of the main coral species, site selection, substrate manufacturing, outboard media manufacturing and transplant shelving. The implementation stage comprised the selection and cutting of the reef, attachment of fragments to substrates, arrangement of fragments in baskets, placement of fragments in transplant shelves and plantings. The observation stage consisted in monitoring the growth of coral fragments that have been planted and was performed once a week, for 3 months, with intensive controls.

The substrate used as a medium for coral addition was made from cement and sand dough with a ratio of 1:2; the construction of the pre-installed fragment is shown in figure 2.

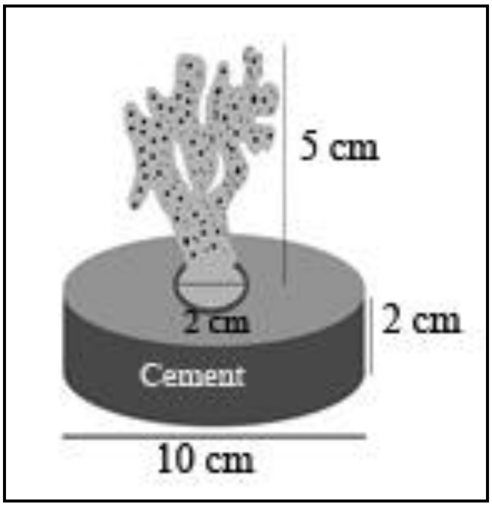

Figure 2. Substrate made from cement and sand dough with coral transplants.

The substrate with corals was set on iron tables. Corals were set up on three transplantation tables of 1×1 m^2 surface. The transplant tables were set approximately 0.5–1 m from the bottom of the water. The water depth of the lowest tide was approximately 1.0–1.5 m. One transplant table contained 32 coral fragments. The first table contained 32 fragments of *P. verrucosa*. The second table contained 32 *S. pistillata* fragments. The third table contained both species, with 16 fragments from each (the mixed transplant table).

The monitoring of coral growth was performed by measuring the vertical growth and colony diameter of the coral fragments and the number of polyps, using plastic calipers (specification: 0.05 mm). Water quality measurements were performed at the beginning and end of the month, for 3 months, in situ, at the depth of the transplanted coral. The measured parameters were: brightness (with a secchi disk), temperature (mercury thermometer), pH (pH meter), current velocity (flowmeter) and salinity (refractometer).

Coral fragments treatment was performed regularly, once a week, to ensure that the transplanted coral fragments did not die and the treatment was performed in line with the observation. The treatment process comprised checking the condition of the coral location on the transplant shelves, the coral health, cleaning of the transplant shelves and checking the condition of the transplant shelves. The falling reef was returned to its original position, the dead coral was discarded or separated from the living coral to minimize the transmission of bleaching and the damaged transplant shelves were immediately repaired.

Statistical analysis. The calculation of the transplanted coral growth was done using the following formula:

$$\Delta L = Lt - Lo$$

Where: ΔL - vertical height or colony diameter increased from coral fragments;
Lt - average vertical height or colony diameter of coral fragments on week t;
Lo - average vertical height or colony diameter of coral fragments at week 0.

The increase in the number of polyps was calculated using formula:

$\Delta N = Ni - No$

Where: ΔN - the increase in the number of polyps;
Ni - the average number corals polyps at the week t;
No - the average number of coral polyps at the initial week 0.

The coral survival rate was calculated by the Ricker (1975) formula:

$SR (\%) = {}^{Nt}/_{No} \times 100$

Where: SR - survival rate;
Nt - the number of individuals coral at the end of the study;
No - the number of individuals at the beginning of the study.

The collected data includes vertical height, colony diameter of coral fragments, polyp number and survival rate in each observation week. The differences in coral growth between single and mixed cultivation and between *S. Pistillata* and *P. verrucosa* were checked using a parametric t test at 5% significance level, using Microsoft Excel 2007 and IBM SPSS version 19.

Results and Discussion

Coral growth. Transplanted coral reefs cultivated on the Serangan coastline displayed vertical and horizontal growth and showed an increase in the number of buds over time. The mean vertical height, colony diameter and the number of polyps cultivated in the single species of *S. pistillata*, *P. verrucosa* and each species in mixed species are presented in Table 1.

Table 1
The initial and final mean length and height of coral fragments planted in Serangan Coastal Waters

Planting	*Species*	*N*	*Initial*			*Final*		
			Colony diameter (mm)	*Height (mm)*	*Polyps (buds)*	*Colony diameter (mm)*	*Height (mm)*	*Polyps (buds)*
Single	*S. pistillata*	32	61.09±11.29	44.5±5.28	10.09±4.72	74.10±11.37	53.39±5.83	26.28±8.57
	P. verrucosa	32	64.13±12.71	48.57±6.75	4.13±1.88	74.41±12.71	54.46±6.95	9.66±4.02
Mixed	*S. pistillata*	16	53.88±8.32	40.96±7.83	9.07±2.28	46.01±8.16	62.63±7.96	18.81±6.41
	P. verrucosa	16	58.03±8.47	44.58±6.97	3.75±1.24	65.92±8.74	65.92±7.53	8.44±2.44

The growth of transplanted corals during the 12 weeks is presented in Table 2. The coral cultivated in the mixture of the two species presented growth deficiencies when compared with the ones in single species cultivation.

Weekly coral growth. The increase in the height of the corals per week is presented in Table 3. When in single species, as well as in the mixed treatment, *S. pistillata* grew better than *P. verrucosa*. In the single species planting, the average growth per week of *S. pistillata* had the value of 0.74 mm, whereas the one of *P. verrucosa* was 0.49 mm. In the mixed planting, the growth of *S. pistillata* was 0.42 mm/week and the one for *P. verrucosa* was 0.30 mm/week. Hence, the coral cultivated in single species presented better growth compared with the mixed planting of the two species.

Table 2
Growth of transplanted corals for 12 weeks after planting in Serangan Coastal Waters

Planting method	*Species*	*n*	*Colony diameter (mm)*	*Height (mm)*	*Polyps (buds)*
Single species	*S. pistillata*	32	13.02±1.73	8.83±2.51	16.19±4.75
	P. verrucosa	32	10.28±1.58	5.89±1.24	5.53±2.54
Mixed	*S. pistillata*	16	8.75±2.35	5.04±1.97	9.75±4.91
	P. verrucosa	16	7.88±2.47	3.49±1.17	4.69±2.09

The average colony diameter of coral fragments per week is presented in Table 4. The increase in the colony diameter of the coral in single species cultivation was greater than that of the mixture of the two species. Whether coral species of *S. pistillata* and *P. verrucosa* were cultivated in single or mixed cultivation, horizontal growth was faster than vertical growth.

Table 3
The mean vertical growth of corals per week (x±standard deviation, in mm)

Week	*Stylophora pistillata*		*Pocillopora verrucosa*	
	Single	*Mixed*	*Single*	*Mixed*
1	0.76±0.51	0.18±0.25	0.62±0.20	0.20±0.24
2	0.67±0.47	0.53±0.23	0.47±0.30	0.53±0.42
3	0.46±0.46	0.43±0.36	0.29±0.28	0.29±0.25
4	0.85±0.52	0.49±0.31	0.58±0.43	0.39±0.40
5	0.51±0.23	0.45±0.38	0.66±0.22	0.60±0.22
6	0.69±0.56	0.47±0.75	0.45±0.53	0.29±0.56
7	0.40±0.44	0.06±0.19	0.46±0.66	0.08±0.22
8	0.50±0.32	0.63±0.70	0.44±0.31	0.07±0.17
9	1.15±0.58	0.52±0.41	0.67±0.60	0.28±0.36
10	0.88±0.53	0.51±0.57	0.34±0.34	0.33±0.49
11	0.92±0.56	0.28±0.44	0.33±0.27	0.08±0.15
12	1.04±0.47	0.48±0.35	0.56±0.35	0.39±0.30
Mean	0.74±0.10	0.42±0.18	0.49±0.15	0.30±0.13

The increase in the number of new polyps is presented in Table 5. The emergence of new polyps in *S. pistillata* was approximately 7 times more than the one in the case of *P. verrucosa*.

Table 4
The average colony diameter (x±standard deviation, in mm) measured weekly

Week	*Stylophora pistillata*		*Pocillopora verrucosa*	
	Single	*Mixed*	*Single*	*Mixed*
1	1.19±0.65	0.88±0.83	0.93±0.32	0.43±0.39
2	0.76±0.31	0.44±0.68	0.71±0.36	0.56±0.60
3	0.99±0.42	1.11±0.82	0.57±0.36	0.61±0.62
4	0.87±0.60	0.54±0.65	0.74±0.40	0.50±0.42
5	0.44±0.22	0.53±0.35	0.67±0.34	0.63±0.58
6	1.74±0.70	1.11±0.99	1.25±0.63	0.89±0.91
7	1.18±0.58	0.95±0.97	1.28±0.60	1.04±1.34
8	0.50±0.30	0.61±0.59	0.91±0.65	0.67±0.66
9	1.98±1.30	0.79±0.91	0.57±0.48	0.68±0.73
10	0.75±0.48	0.83±0.72	0.99±0.59	0.76±0.84
11	1.32±0.72	0.61±0.84	0.83±0.57	0.86±1.07
12	1.29±0.36	0.48±0.45	0.84±0.51	0.57±0.57
Mean	1.08±0.29	0.74±0.20	0.86±0.12	0.68±0.27

The survival rate of the corals was determined as percentage (%). Both *S. pistillata* and *P. verrucossa* resulted in a 100% survival rate during the experiment transplantation in single and mixed species.

Table 5

The average number of increase in coral fragments polyps (buds) per week

Week	*Stylophora pistillata*		*Pocillopora verrucosa*	
	Single	*Mixed*	*Single*	*Mixed*
1	90±4.78	19±2.82	8±2.12	2±1.24
2	65±4.66	16±3.05	22±2.51	3±1.39
3	165±4.81	28±3.68	7±2.43	0
4	3±4.84	1±0.38	4±2.34	1±1.26
5	36±4.94	10±3.61	14±2.37	4±1.24
6	191±6.39	51±5.39	12±2.60	2±1.18
7	252±7.33	0	12±2.73	0
8	155±7.08	10±5.57	32±3.42	0
9	174±8.13	19±6.02	26±3.67	4±1.45
10	162±8.08	28±5.91	37±3.81	12±1.86
11	155±8.27	19±6.18	34±4.20	7±2.09
12	226±8.57	26±6.41	34±4.02	10±2.44
Average	139±75.66	19±13.62	19±11.58	4±4.03

The coral growth differences were tested for the single and mixed planting methods, and for both species in each method, the results being presented in Table 6. There were statistically significant differences (t test, $p<0.05$) in the vertical growth, colony diameter and emerging new polyps of single and mixed planting methods. However, the mixed planting for both species showed no significant differences in vertical growth and colony diameter.

Regarding the horizontal and vertical growth of the coral colonies, there were statistically significant differences in the diameters of coral colonies in single and mixed planting methods. The number of branches of coral fragments was statistically significant in differences between *S. pistillata* and *P. verrucossa* planted in single and mixed methods (t test, $p<0.5$).

The physical and chemical parameters of the water were measured near the cultivated coral reefs from October 2016 to January 2017 and are presented in Table 7. Generally, the physical and chemical conditions of the aquatic environment were within the optimum range for coral reefs. The water temperature ranged from 28°C to 31.33°C, whereas the pH value ranged from 7.5 to 8.0. The water turbidity was low enough for the sunlight to penetrate the bottom of the water. The salinity ranged from 32.83 to 35.33 ppt and the current velocity was between 0.03 and 0.14 m/s. The water quality was relatively stable to support the growth of coral reefs.

In this study, the coral transplants cultivated both in single and mixed species increased in height and colony diameters over time. In addition, fragments also increased with respect to the number of branches and the emergence of new polyps indicate that they could grow on substrates from the Serangan coastal area. Generally, *S. pistillata* grew in height and colony diameter by 0.74 mm/week and 1.08 mm/week, respectively, whereas *P. verrucosa* grew in height and colony diameter by 0.49 mm/week and 0.86 mm/week, respectively. Compared to other studies that used different species from the Seribu Island, this growth was relatively slow. It was reported that *Acropora formosa* grew in length by 0.90–1.61 mm/week (Fadly 2009).

Table 6
The coral growth differences (t test) of *S. Pistillata* and *P. verrucossa* in the single and mixed planting

Growth	*Species*	*Planting*	*N*	*Mean*	*Comparison*	*t*	*P*
Height	*S. pistillata*	Single	32	0.4906	SP vs SV	4.762	0.000*
		Mixed	16	0.2937	SP vs MxP	5.099	0.000*
	P. verrucossa	Single	32	0.7366	SP vs MxV	1.569	0.124*
		Mixed	16	0.4219	SV vs MxP	6.585	0.000*
					SV vs MxV	4.472	0.000*
					MxP vs MxV	2.765	0.010*
Colony Diameter	*S. pistillata*	Single	32	0.8575	SP vs SV	4.279	0.000*
		Mixed	16	0.6606	SP vs MxP	2.912	0.006*
	P. verrucossa	Single	32	1.0850	SP vs MxV	1.904	0.063*
		Mixed	16	0.7288	SV vs MxP	7.278	0.000*
					SV vs MxV	4.472	0.000*
					MxP vs MxV	1.002	0.324
New Polyps	*S. pistillata*	Single	32	6.2131	SP vs SV	9.407	0.000*
		Mixed	16	5.2263	SP vs MxP	1.380	0.174*
	P. verrucossa	Single	32	16.863	SP vs MxV	7.264	0.000*
		Mixed	16	13.692	SV vs MxP	7.975	0.000*
					SV vs MxV	1.918	0.061*
					MxP vs MxV	7.328	0.000*

Note: SP - *S. pistillata* single planting; MxP - *S. pistillata* mixed planting; SV - *P. verrucossa* single planting; MxV - *P. verrucossa* mixed planting. * - significant difference p<0.05.

The horizontal growth rates of *S. pistillata* or *P. verrucosa* were greater than the vertical growth rates. Some factors that have an effect on horizontal growth are the depth of water, cultivation and water current. The depth of water for the cultivation of *S. pistillata* and *P. verrucosa* was less than 3 m and the water was clear; therefore, sunlight penetrated to the bottom of the water. Generally, organisms that have chlorophyll will grow towards sunlight; hence, growth in the clear water area is be better. Similarly, zooxanthella, including chlorophyll algae in symbiosis with coral animals, will always require sunlight for photosynthesis. Therefore, near *S. pistillata* or *P. verrucosa* fragments there will be a much larger density of algae that grow faster. The presence of strong currents cause *S. pistillata* or *P. verrucosa* to grow broadly. Generally, corals living in areas with a strong current will present a form of growth with thick buds and flat ends (Rachmawati 2001). Corals exposed to high waves with an intense penetration of sunlight will have shorter and blunted shoots growth. *S. pistillata* and *P. verrucosa* have a form of corymbose growth (Veron 1986), or they grow in the form of large fingers.

Coral reefs display symbiosis with zooxanthellae. Corals regulate the symbionts in three ways: by the release, installation and inhibition of zooxanthellae growth. The distressed coral reefs will regulate the symbionts; hence, when the reefs are distressed, they will tend to release the zooxanthellae and the growth process will be hampered (Hoegh-Guldberg & Smith 1989). Many factors stress living corals, like environmental factors, including temperature, salinity, flow or sedimentation, and other factors, such as competition with other coral species that live close together. The competition with other coral reefs refers to a better nutrition or space as a place to live. Competition may be observed in the form of a toxic substance released from the coral body, a growth pattern that covers the growth of other species or with a stinging cell (nematocyte) to kill other co-existing species.

Table 7

Water quality observed during cultivation of transplanted coral in Denpasar

Week	*Temperature (°C)*	*pH*	*Salinity (ppt)*	*Current (m/sec)*
1	31.0	8.0	35.3	0.07
2	31.3	8.0	33.7	0.11
3	31.1	8.0	34.3	0.08
4	31.2	7.5	34.3	0.08
5	30.0	8.5	34.3	0.10
6	30.0	8.5	34.3	0.07
7	29.2	8.0	33.5	0.04
8	28.0	8.5	32.8	0.03
9	31.3	8.0	32.8	0.05
10	30.5	8.5	34.7	0.14
11	30.7	8.0	34.7	0.10
12	30.8	8.5	34.7	0.08

Corals *S. pistillata* and *P. verrucosa* that were cultured singly or as mixed species displayed experience competition. In the single coral reef cultivation, the competition is among the same species, whereas in the mixed cultivation, the competition is among the same and different species to obtain feed, space, light or other resources. Corals that are able to use more diverse types of nutrients, synthesize nutrients more efficiently and are able to adapt to the environmental changes have better growth and resilience. The species *S. pistillata* has more polyps and longer fragments than *P. verrucosa*, so *S. pistillata* has a wider surface to take more nutrients.

Water quality and environmental conditions played an important role in meeting the nutritional needs of coral reef transplants cultivated in the coastal area of Serangan Island. The water quality in the Serangan Island area met the conditions for growing and living coral reefs. The water temperature interval was between 26°C and 28°C (Birkerland 1997). Coral reefs need a pH between 6.5 and 8.5, as acid-tending waters will decrease the ability of corals to produce $CaCO3$ (Kleypas & Kimberly 2009). The water acidity in the Serangan Island area tends to be alkaline, so it was within the tolerable range for coral reefs. Another important water parameter is the optimum salinity for coral growth, ranging from 32 to 35 ppt (Nybakken 1992). The average salinity was between 32.83 and 35.33 ppt, being ideal for coral growth. The presence of strong currents was useful for naturally cleaning coral polyps from marine material deposits, so that the lifespan of coral reefs transplantation was long (Birkerland 1997).

The survival rate describes a successful transplant performed on the reef. The coral transplantation of *S. pistillata* and *P. verrucosa* at the Serangan Beach is successful because the survival rate reaches 100%. The high survival rate shows that the conditions met in Serangan Coast waters are favorable for coral transplantations.

Conclusions. Coral reef transplantation of *S. pistillata* and *P. verrucossa* in single species grow faster and better than in the mixed transplantation of the two species. *S. pistillata* displayed better and faster growth than *P. verrucossa*. The coral colony of *S. pistillata* displayed an increase in new polyps, the number being higher than in the case of *P. verrucossa*. The conditions of the aquatic environment for transplanting corals were more than acceptable and able to support the life of coral reefs. All transplanted reefs cultivated in single species or mixed live well and have a high survival rate.

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