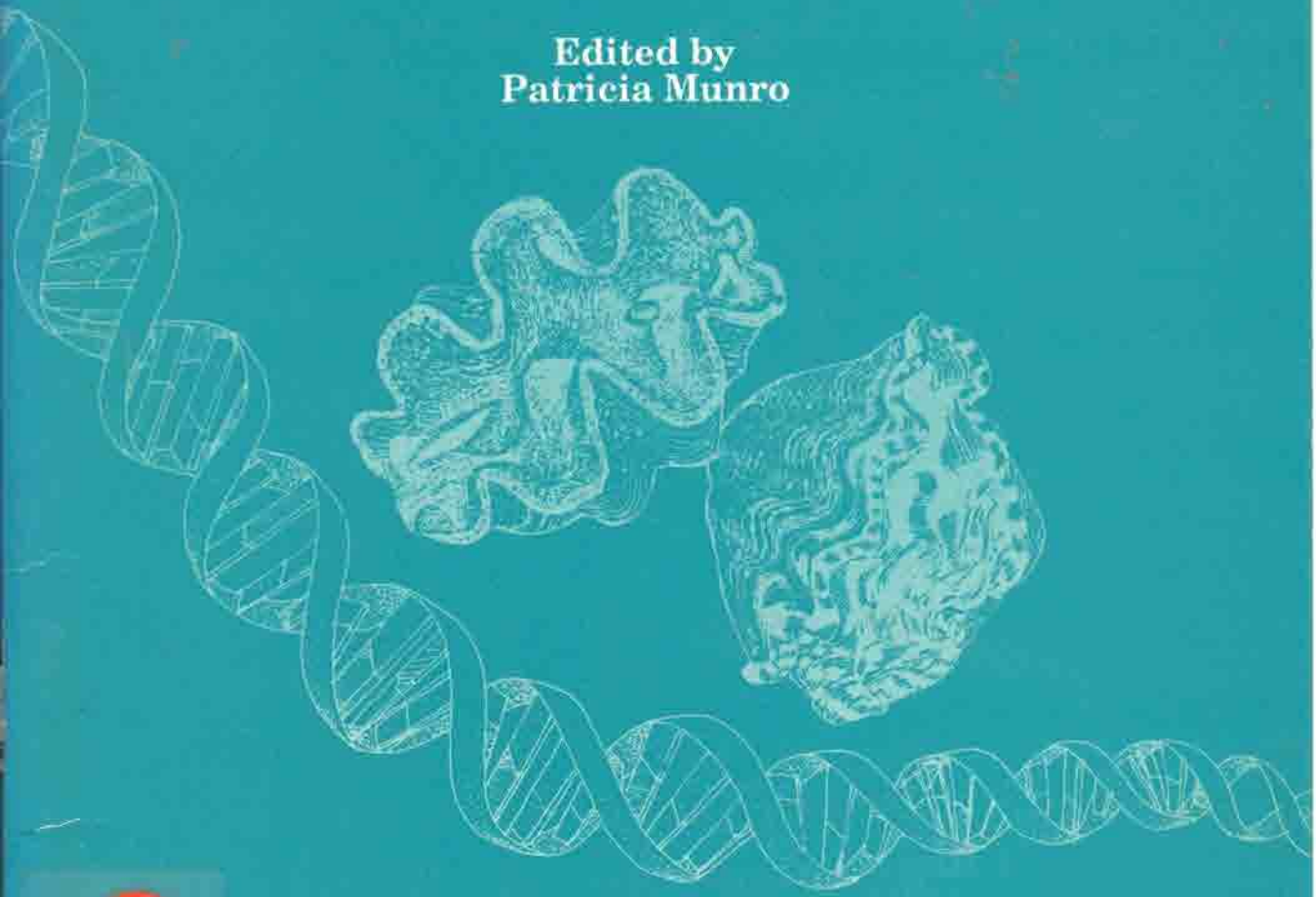
Genetic Aspects of Conservation and Cultivation of Giant Clams

Report of the Workshop
held on 17-18 June 1992
at the ICLARM Headquarters
Makati, Metro Manila, Philippines

Edited by
Patricia Munro



Australian Centre for International Agricultural Research

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International Center for Living Aquatic Resources Management

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Printed in Manila, Philippines

Published by the International Center for Living Aquatic Resources Management Coastal Aquaculture Centre, P.O. Box 438, Honiara, Solomon Islands; the Australian Centre for International Agricultural Research, GPO 1571 Canberra, ACT 2601, Australia; and the International Development Research Centre, IDRC BP 8500 Ottawa, Canada K1G 3H9.

Munro, P., Editor. 1993. Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

ISBN 971-8709-36-3
ISSN 0115-4435

ICLARM Contribution No. 914

Contents

Preface

Anyone who has dived or snorkelled on coral reefs in the Indo-Pacific has enjoyed the sight of a giant clam, brightly colored mantle open to the sunlight shining through the clear warm water. Unfortunately in many areas giant clams are now extinct, or nearly so. The reason for this is not hard to understand; giant clams are easily harvested and accessible to the least intrepid of gatherers. What may seem to be a somewhat esoteric subject for aquaculture is a highly esteemed food item in all parts of the tropical Pacific. In the culture of Pacific Islanders, giant clams have great traditional significance, which is difficult to convey to outsiders.

Cultivation of giant clams has been established in many countries, and extinction of the species is now unlikely. However in many places some species are no longer there at all, or in such small numbers as to be nonviable. Transfer of stocks of clams grown or found in one place to another has certain genetic and ecological consequences, as well as being a possible mechanism of disease spread. For some time ICLARM has been foremost in warning of the possible consequences of transfers and introductions, not only of tridacnids but other organisms.

ICLARM's role in convening the Giant Clam Genetics Workshop was to promote regional cooperation in breeding giant clams, and provide a forum for discussion of the re-establishment of stocks in a genetically sound way. Conservation of genetic resources is not simply conservation for its own sake, but the cheapest and most effective way of developing a biological asset.

Participants invited to the workshop included scientists involved in the Giant Clam Research Group of ICLARM's Coastal Aquaculture Network, and geneticists from Australia, Canada and ICLARM headquarters. Funding was provided by ACIAR, IDRC, ICOD, ODA and ICLARM. There was an awareness that as giant clam farming is in its infancy, a unique opportunity exists to avoid the mistakes made in older, established aquaculture enterprises such as salmon farming, as well as to learn from their successes. As in all breeding programs which start with a wild stock, enormous gains can be expected by selection of desirable traits within a few generations.

The proceedings of the workshop consist of discussion papers presented by John Benzie (AIMS), Gary Newkirk (Dalhousie University), John Munro (ICLARM), Mark Gervis (ICLARM), and Julie Macaranas (Queensland University of Technology, formerly of UPMSI), subsequent discussions at the workshop, and a series of country papers presented by delegates from the Philippines, Australia, Solomon Islands, the Federated States of Micronesia, Palau, and Fiji.

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Acknowledgements

We wish to acknowledge the support of the Australian Centre for International Agricultural Research, and the International Development Research Centre of Canada in providing funding for participants to attend the Workshop, and for publication of these proceedings. We are grateful to the International Centre for Ocean Development and to the Overseas Development Administration of the United Kingdom for providing funding for some participants. The active participation of the various institutions involved is also acknowledged with appreciation.

List of Abbreviations

ACIAR	Australian Centre for International Agricultural Research
AIDAB	Australian International Development Assistance Bureau
AIMS	Australian Institute of Marine Science
CAC	Coastal Aquaculture Centre
CITES	Convention on International Trade in Endangered Species
FON	floating ocean nursery
FSM	Federated States of Micronesia
GBR	Great Barrier Reef
ICES	International Council for Exploration of the Sea
ICOD	International Centre for Ocean Development
IDRC	International Development Research Centre
IUCN	International Union for the Conservation of Nature
JCU	James Cook University
MMDC	Micronesian Mariculture Demonstration Centre
MPA	marine protected area
ODA-UK	Overseas Development Administration of the United Kingdom
OIRS	Orpheus Island Research Station
SL	shell length
SUML	Silliman University Marine Laboratory
UPMSI	University of the Philippines Marine Science Institute

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Review of the Population Genetics of Giant Clams*

JOHN A.H. BENZIE, *Australian Institute of Marine Science, PMB No. 3, Townsville, Qld. 4810, Australia*

BENZIE, J.A.H. 1993. Review of the population genetics of giant clams, p. 1-6. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction

Surveys of genetic variation have been undertaken of the two species of greatest economic interest, *Tridacna gigas* and *T. derasa*, throughout the western Pacific (Ablan et al. 1993; Benzie and Williams 1992a; Macaranas et al. 1992). However, these species have become rare or extinct over large parts of their range due to overexploitation, and sampling was necessarily patchy. In order to understand better the patterns of variation that might emerge, surveys were also undertaken of *T. maxima*, a smaller species that is widespread throughout the Indian and Pacific Oceans, and for which a greater geographical coverage was expected (Benzie and Williams 1992b). The aim of this paper is to summarize the findings of this recent work.

Allozyme variation has now been examined in several hundred individuals of *T. gigas*, *T. maxima* and *T. derasa* from wild populations throughout the Pacific. All surveys used biopsies of mantle tissue that allowed clams to be sampled *in-situ* without sacrificing them. Summaries of the techniques used are discussed by Dr. Macaranas (this vol.); (Benzie et al. 1993).

Populations in each species clustered together consistently as follows: the GBR, the Philippines and the Solomon Islands, first cluster together, followed by Fiji and Tonga as outliers, in a 'West Pacific' group. Samples from the Cook Islands, Kiribati and the Marshall Islands form a separate 'East Pacific' group. F-statistics were used by each study to partition genetic variation into that occurring within populations (F_{IS}), and that occurring between populations (F_{ST}). No study found significant structuring within populations, and all reported general conformance of gene frequencies to those expected under conditions of random mating (conditions of Hardy-Weinberg Equilibrium). All reported little differentiation among populations within local regions such as the Solomon Islands or highly connected reef systems such as the GBR, but all species showed significant differences among populations on greater geographical scales (Table 1).

The pattern of gene flow among clam populations showed remarkable similarities among species, and demonstrated clearly that the increasing significance of population differentiation at the regional level was not simply the result of increasing genetic divergence with increasing geographical separation (Fig. 1). Fiji was as isolated from neighbouring Kiribati as it was from the Philippines. Gene flow was very high within local areas (usually $N_em>20$) and for *T. gigas* and *T. maxima* relatively high between the Philippines, the GBR and the Solomon Islands ($N_em>10$). There appear to be major barriers to gene flow between the East and West Pacific groups ($N_em<2$), and east-west between Australia, the Solomons, Fiji, Tonga and Micronesia. The greatest connections follow the island chains connecting the Philippines through New Guinea to Australia, and separately to the Solomon Islands. These patterns of gene flow are similar to biogeographical patterns of distribution of marine faunas (Springer 1982), suggesting a fundamental structuring of giant clam species. It is not known whether these patterns reflect

*Contribution No. 821 from the Australian Institute of Marine Science.

Table 1. Genetic differences among populations in different geographical regions (all values are F_{ST}, which describes genetic variation occurring among populations). F-statistics were calculated using methods which explicitly take account of differences in sample sizes among the populations tested, and their significance was tested using chi-square (Waples 1978). Data abstracted from Benzie and Williams [1992a] and calculated from data in Ablan et al. (1993) and Macaranas et al. (1992).

	T. gigas	*T. maxima*	*T. derasa*
WITHIN LOCAL AREAS			
GBR	0.000^{ns}	0.003^{ns}	0.012^{ns}
Solomon Islands	0.011^{ns}	-0.003^{ns}	-
Philippines	-	-0.002^{ns}	-
Kiribati	-	-0.003^{ns}	-
WITHIN REGIONS			
East Pacific	0.032*	0.068***	-
West Pacific	0.035***	0.099***	0.098***
All populations	0.084***	0.156***	0.098***

* P<0.05 *** P<0.001 ns - not significant

a continuing pattern of dispersal present day, or reflect historical fluxes of migration that no longer occur.

Samples of 90 individuals from each of three hatchery batches from both the Solomon Islands and the GBR revealed lower average levels of genetic diversity within hatchery stocks of *T. gigas* than the natural populations from which the broodstock was derived (Table 2). This was not surprising in that very few individuals were used to produce each batch, and it was thought that the Solomons families were the product of single matings. The occurrence of more than four alleles for a given locus at a number of systems demonstrated clearly that more than two parents were involved in the production of each of these batches.

Gene frequencies of the cultured stocks were markedly different from the native populations, giving greater genetic distances among cultured batches, and between cultured batches and natural populations, than among any of the natural populations (Fig. 2). Indeed, the level of differentiation among cultured batches was similar to that between populations from different regional groups (i.e., West and East Pacific). No significant correlations were observed for *T. gigas* between size at a given age within a batch and specific genetic markers or with heterozygosity [Benzie and Williams, unpubl. data].

Discussion

The only published data available on giant clam genetics prior to the recent studies concerned two populations of *T. maxima*, one from the Marshall Islands and one from the GBR (Campbell et al. 1975). They found small genetic differences over 4,000 km suggesting considerable dispersal by giant clams throughout the Pacific. Under these circumstances, transfers of live material throughout the Pacific might be considered useful enhancements of local stocks by genetically similar introductions, irrespective of their source.

The recent studies, specifically aimed at analyzing population structure, have provided powerful evidence of fundamental genetic structuring of giant clam populations in the Pacific. The few large populations of giant clams that exist and which could be used as a source for broodstock differ in genetic constitution (e.g., GBR and Micronesian populations of *T. gigas*). The source of material to be transferred to a location is now a critical issue if the aim is to enhance local stocks without endangering local genetic diversity. A revision of hatchery techniques will be required to produce genetically diverse batches. Restocking programs may require several introductions over time, and include the progeny from many matings in order to produce populations whose gene frequencies approach those of natural local stocks.

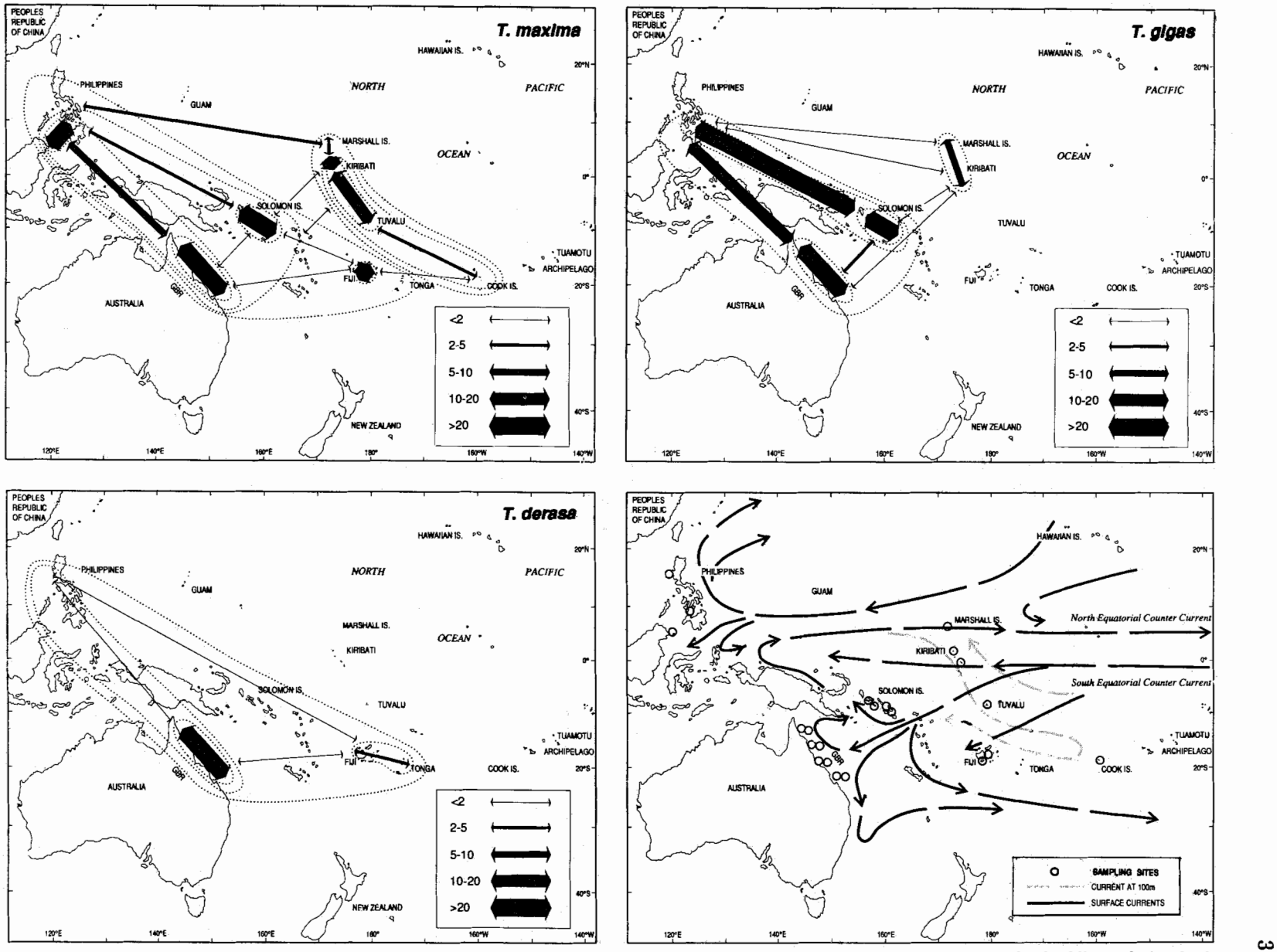

Fig. 1. Gene flow among *Tridacna gigas*, *T. maxima* and *T. derasa* in the populations in the West Pacific. The thickness of the arrows represents different levels of dispersal, given by the average number of migrants per generation (N_em). N_em is the average number of migrants per generation calculated from F_{ST} as follows: $N_em = ((1/F_{ST})-1/4)$. Pairwise comparisons of population groups were made after pooling all the populations within each group so that no within-group component of gene flow was included in the between-group estimate.

Table 2. Average genetic diversity in cultured batches of *T. gigas* compared with wild populations from the same region, where possible. Cultured batches from the GBR and the Solomon Islands were about one year old and were still in the hatchery or in ocean growout nearby. Those from Palau were about two years old and had been translocated to reefs in Kosrae. Comparisons used eight loci for which data were available for both cultured and wild populations.

	Great Barrier Reef		Solomon Islands		Palau
	Wild	Cultured	Wild	Cultured	Cultured
Mean number of alleles per locus	2.0 (1.8-2.1)	1.6 (1.4-1.8)	2.2 (2.0-2.3)	2.0 (1.8-2.0)	1.6 (1.6)
Percentage of loci polymorphic	50 (38-63)	38 (25-50)	53 (50-63)	50 (50)	38 (38)
Direct count hetero-zygosity	0.20 (0.19-0.22)	0.16 (0.10-0.20)	0.30 (0.25-0.36)	0.27 (0.18-0.34)	0.36 (0.35-0.36)
No. of populations or batches screened	6	3	4	3	2
No. of individuals screened per population	57-74	90	9-37	90	20-30

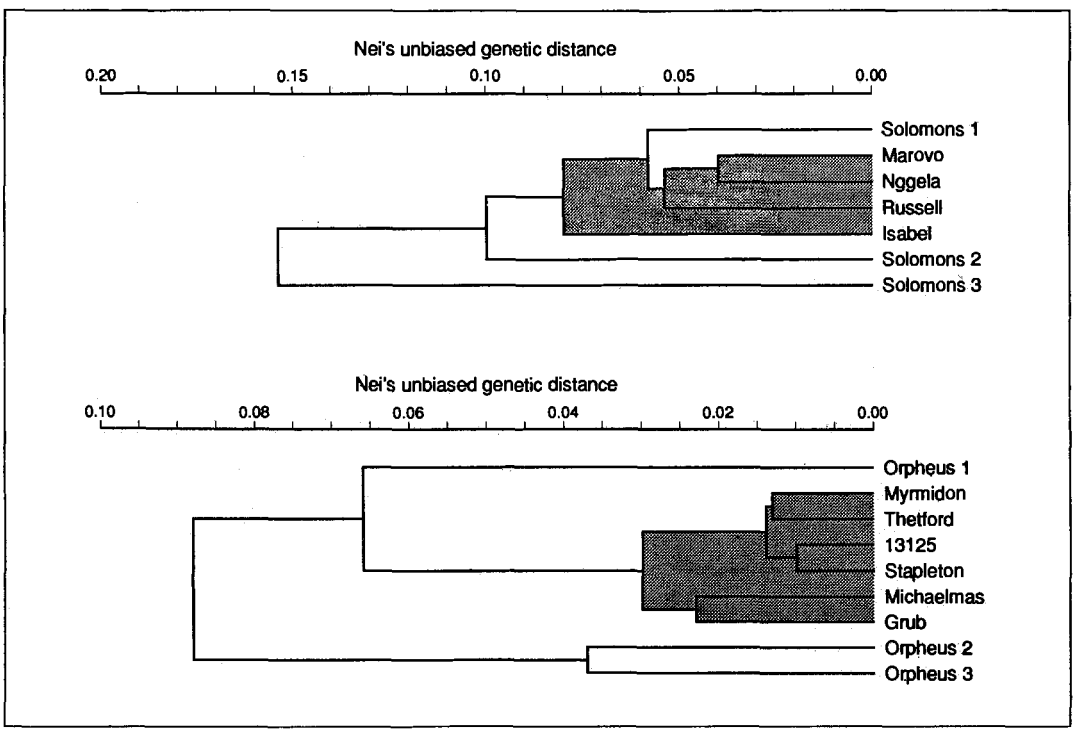

Fig. 2. Dendrograms illustrating the considerable genetic divergence among cultured batches relative to each other and to the natural populations from which they were derived.

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Discussion

J. MUNRO: In relation to gene flow, you said that material could have come from the Philippines to the Solomons. On oceanographic grounds one would expect material to have come from the Solomons to the Philippines?

BENZIE: You can't tell the direction of flow from the genetic data alone.

J. MUNRO: The pattern that you have shows a flow going from the Torres Straits say, up through Indonesia and getting entrained in the South Equatorial current which goes through the Solomons along the north coast of New Guinea and straight to the Philippines. That would make a lot of sense.

BENZIE: It would. But equally if you look at major surface currents, they trend east to west, so that the limited gene flow that occurs between those groupings is apparently at variance with the major surface flow. If you were simply to look at currents, it would be quite likely that you would get transferral between the Cooks and Tonga, and Tonga and Fiji. Now that clearly doesn't occur. I can't distinguish between gene flow that might be occurring now and gene flow that occurred a long time ago and no longer occurs. In terms of biogeographical patterns in the Pacific, the patterns of various species distributions and the hiatus in a great many species distributions, there seems to be a major genetic break which is parallel to the Pacific plate margin. So we're not sure whether we're looking at dispersal patterns coming through from the western Indo-Pacific and moving eastwards, or whether some of the differentiation is the result of populations which have been separated much longer. There's no way from these data to tell.

PULLIN: Where you've only got a small population of clams surviving across this range, or even across a wider geographical range, this may be a unique point at which to sample these clams, or even to try to transplant some of them and keep them somewhere. Once captive support breeding programs start, or farming starts, the nature of a wild type population will change. IUCN and others are thinking about this for some of their captive support breeding programs now.

EKNATH: What is the time scale for the divergence? How long have they been isolated to come up with this low level of heterozygosity?

BENZIE: There's no particular time scale identified. These animals are very highly heterozygous.

NEWKIRK: If the parent animals came from the wild, and the larvae were produced in the hatchery, then I think what the data are indicating is something about the sampling procedure, and nothing really about cultured vs. wild stocks.

BENZIE: These larvae may be used to restock reefs and to stock farms, and this is the sort of genetic material that one might expect to be produced in the hatcheries.

NEWKIRK: There appear to be small differences in the numbers. But I think the basis is in the small number of batches that you've looked at, and if you did look at all of the batches in these hatcheries throughout a year or two, the genetic results from that kind of sampling would be more similar to those of the wild.

BENZIE: If all the batches were used we might come toward the mean. It's sometimes difficult to get the animals to spawn. But I don't really have any argument with what you're saying.

J. MUNRO: To date all the batches have been produced from wild parents, and except perhaps in Palau, none of these things has reached maturity yet. In the case of *T. gigas*, all of the economic projections put the optimum size of harvest below the size of female maturity. So it seems likely that in a farm situation *T. gigas* would never be reared to female maturity and there would be no impact on the wild stocks. I think this is an area which we need to explore in more detail.

MACARANAS: Based on this picture of population structure, could you say something about realistic management units at this point?

BENZIE: If you mean operational areas which you may wish to protect, I would certainly say the east and west Pacific, and the Solomons and the GBR. I'm concerned about the lack of gene flow within the western Pacific, and within the eastern Pacific between some of the island groups. You'll note that the degree of flow between the Cooks and Kiribati is also quite small. I'm not quite decided about how one might deal with that situation. But certainly there's a major difference between east and west Pacific, and that may be derived from ancient events which are unlikely to be repeated. They constitute extremely important resources.

A Discussion of Genetic Aspects of Broodstock Establishment and Management*

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NEWKIRK, G. 1993. A discussion of genetic aspects of broodstock establishment and management, p. 6-13. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction

In discussing aquaculture many people refer to the great potential of selection in improving stocks. The present status of our aquaculture stocks might be compared to the wild jungle fowl prior to domestication. Improvements in the production of broilers and egg producing chickens raise hopes of similar improvements in aquaculture species. If we are to make similar progress in the genetic improvement of giant clams, a very clear effort is necessary to establish selective breeding programs based on sound animal breeding techniques.

Although we think of genetic programs as being long term and may hesitate to invest in them, a significant economic return may be forthcoming in the moderate term, i.e., a few generations. Furthermore, improper genetic management of broodstock can create a deterioration of performance and thus a loss in production. A possible few extra per cent improvement each generation will make a big difference in several generations. Proper broodstock management will maintain the maximum rate of improvement and will avoid the problems of inbreeding.

There is no magic in a selection program, it is a steady process, a gradual improvement of the stock. There are few shortcuts even with well-established agricultural stocks. The new DNA technologies can not be used effectively in a species which is still wild, and where they can be used they must be accompanied by traditional breeding programs.

This paper is a discussion of the genetic principles of establishing and maintaining stocks for aquaculture. Specifics regarding the status of giant clam hatchery stocks, the wild population structure and the logistical or environmental problems of transferring stocks were discussed during the workshop and some suitable means to include sound genetic husbandry methods in establishing and maintaining giant clam hatchery stocks are mentioned here.

Broodstock Establishment

Before the first animal is obtained a careful evaluation should be made of the potential

*An abridged version of this paper appeared in the Newsletter of the Giant Clam Research Group, Clamlines 11, December 1992.

sources of stock. Giant clams are wild and there has been very little, if any, scope for domestication. If a stock is maintained in culture for a few generations, we can anticipate that there will be at least natural selection to adapt the animals to the new (culture) environment. There will also probably be artificial selection by the culturist. Thus, there may well be, in fact we hope there will be, genetic change. Once the process of domestication and/or genetic improvement has started any introduction of wild stock will be retrogressive. Thus, it behooves us to plan carefully the initial formation of the broodstock so there is sufficient genetic diversity and a concentration of genes from the most appropriate source(s).

There are usually a large number of populations to serve as sources of stock. Whether these natural populations are genetically different and can provide different genetic stocks for breeding purposes depends on a number of factors. Environmental differences may be sufficient to have caused different selection pressures and consequently different genetic adaptations. Or, natural populations may be genetically isolated to varying degrees as a result of geographic separation. This will enhance the genetic differentiation brought about by natural selection.

Human activities in transplanting stocks, particularly in restocking programs, may break down and eliminate the natural genetic differences between populations. Depletion of natural stocks and subsequent re-establishment either by human or natural processes will result in reduced genetic differentiation. The re-establishment of populations may be with a small number of parents which will affect the differentiation of populations randomly but will cause a reduction of genetic variance within the populations.

In choosing sources of stock, the most relevant information is that on the performance of the stock in a culture environment similar to the target environment. If there is very little or no information as guidance in choosing stocks there are several approaches that can be used:

First, a single stock based on whatever information is available can be chosen. This can be risky if the information is incomplete. Taking all stock from one source is "putting all your eggs in one basket".

Second, one can take a number of stocks and do performance evaluation during the first generation. This will require maintaining stock identity and performance records. This approach will be discussed in more detail below.

The third approach is to cross animals from different populations to form a mixed base population. This can be done if parents from a number of stocks can be spawned at the same time. Little information will result on the relative merits of sources but the resulting progeny should be genetically heterogeneous. (The level of genetic heterogeneity in the offspring will depend on the number of parents used and the genetic differentiation among the source populations.)

For the hatchery that intends to maintain and improve its own stock, consideration of the source of stock is extremely important. Such a hatchery should consider taking the second approach: obtaining several stocks, maintaining stock identity and evaluating the stocks. However, limited hatchery sources of stock will restrict the choices.

One must then decide how large a sample of parents to take and what kind of mating scheme to use. The more parents sampled in the initial spawning the greater will be the sample of genotypes included in the stock. In the following generations the offspring of these initial parents will be bred together thus raising the possibility of inbreeding in a few generations. With sufficient numbers of parents initially and control of the stock this problem can be avoided. There is no simple cut off point for "sufficient numbers". The effects of inbreeding decrease with increasing numbers and the genetic diversity increases with increasing numbers. Both of these effects can be calculated (and probably should be for each case). However, generally it would be recommended that there should be a minimum of fifty parents of each sex in each generation. Having less than this is courting trouble; more would be desirable. The numbers can be increased by spreading the spawnings out over time, even to different years as long as there is a regular crossing among groups within each generation.

The initial broodstock should be taken from several different stocks if possible. Unless there is information to suggest favoring one or two particular populations there should be

approximately equal numbers of parents and offspring from each population source.

The next question is what kind of mating scheme should be used. The most desirable approach is to have individual families (single males crossed with single females) maintained through to maturity. In this way when selection of the parents of the next generation is done one can be sure of parentage and avoid mating brothers and sisters. In subsequent generations information taken on parental performance can be used to evaluate individual merit.

For breeding purposes one should isolate a number of spat from each family and grow them as separate families until they are large enough to be individually labeled. When it comes to selecting parents for the next generation one hundred individuals from each family will be plenty for most situations. Selection may occur before sexual maturity, at least female maturity. After selection a reduced number per family is satisfactory. In principle, each broodstock animal should be replaced in the next generation by its offspring. Thus, one actual spawner is needed for each parent the previous generation, once a stable number of broodstock has been reached. One can work backwards to estimate the number of spat that need to be isolated initially using expected survivorship. Thus, even though a female may produce several million eggs and hundreds of thousands of spat, only a few need to be maintained isolated. The rest can be bulk-reared for commercial production.

When fertilization is external as in giant clams there is tremendous flexibility in the kinds of crosses that can be made. For example, one individual can be crossed with many others all at the same time. This means that a great variety of families can be produced from a small number of parents. One reason for having multiple crosses is that some families will be lost. If there is only one mate for each individual the contribution of two parents is lost for each family lost. However, increasing the number of families increases the work.

If single pair families cannot be maintained to maturity some compromises can be made. For example, one individual (as male) can be crossed with two others (as females) and the eggs (or spat) combined after being sure they are viable. This may be extended to more than one male and more than two females. It may be one female crossed with multiple males. In any case, the groups (it may not be appropriate to call them families) should be kept separately identified.

Each time we combine families, eggs or sperm we are losing information and control of the stock. Combining families means one is no longer positive about an individual's parentage. This reduces the flexibility in the matings to be made in the next generation and may lead to inbreeding. However, it is better to lose information and include more genotypes in the initial stock and subsequent generations than to have good control over a smaller gene pool!

If multiple spawners can be induced at one time, the mass spawning approach can be managed such that inbreeding is reduced (to be discussed on p. 10). When clams of different stocks are used to establish the broodstock it will be best to use an individual only once, either as male or female but not both. This will eliminate self-fertilization.

From the information presented at the workshop it seems that all hatcheries induce what the geneticist would consider small numbers, and that there are three types of hatcheries that have been operating, with respect to access to indigenous stock and hatchery methods:

1. large numbers of indigenous stock and mass spawning;
2. large numbers of indigenous stock and few spawners;
3. few or no indigenous stock and few spawners.

These three possibilities will be referred to as: Large-mass, Large-few, and None-few and their roles in re-establishing wild stock and in farming will be discussed.

The Large-mass hatchery is probably the most important type as a source of stock for other places. Though their local population sources may be limited to one large population the fact that they can produce hatchery stock with large numbers of spawners means that the offspring will have as close to natural levels of genetic variation as possible. When None-few hatcheries import stock the early shipments may dominate the broodstock in subsequent years and it will be important to have high levels of genetic variation in these groups. The Large-few hatcheries will be important sources of genes from other natural populations.

However, care will be needed in integrating these stocks into a new broodstock as the batches received may consist of closely related individuals. The None-few hatcheries will initially be mostly receiving stocks but may be sources of stock in the future.

Giant clam stocks will be transferred for two basic reasons: either to produce broodstock for farming or for re-establishing natural populations. Some importers may want stock for both reasons. The simpler situation is the supply of stock for farming as the questions of source of stock and impact on indigenous species are difficult in re-establishment. The main problem is to provide enough genetic variation in the broodstock to allow for natural and artificial selection.

The source of stock will be determined primarily by the availability of seed from existing hatcheries. It is not easy to collect animals from places of choice and move them to hatcheries for seed production. Among the few existing hatcheries a choice may be possible based on the location and types of environment. There is insufficient genetic information available for sound choices among alternative stock sources. The population genetics information (see Benzie, this vol., p. 1) can be used as a guide which indicates general areas where it is thought that gene flow is higher. The implication of this is that the genes for local adaptations may also be more similar between areas of higher gene flow than between areas of low gene flow. However, this information can only be used as an approximation.

Other information may be of more importance. If the habitats of stocks differ, the stock of choice would be the one from a habitat similar to the one where the stock will be raised.

One should consider the potential environmental impact. If there is a local population I presume that the reason for importing more stock is that the local population is almost extinct. Otherwise it is recommended that the local stock be used. If the local stock is very small, one might consider them as being virtually extinct and not worry about the introduction of exotic genes. This will be discussed further with respect to re-establishing stocks.

If the local stock cannot provide sufficient numbers of broodstock to establish the gene pool for a farm broodstock, importations will be needed. Whenever possible, local stock should be incorporated into the broodstock as they probably have genes for local adaptations. The problem is the trade-off between including the local genes but not wanting the broodstock to be based on, or dominated by, a few individuals. The best approach would be to use the local animals in crosses with imported stock and not cross locals with locals. The total number of broodstock used will have to be determined and the general guidelines of using as many as possible should be followed. Since the logistics of giant clam breeding may not allow the numbers a geneticist would like to include (over 50), it is a problem of trying for as many as possible by using every opportunity and assessing the situation after a few years. This will mean using the local animals as much as possible but keeping good records of when they spawn and what juveniles are produced.

As much control as possible should be used. The maximum control is attained by mating two individuals at a time. However, this may not be easy. Mass or small group spawnings are quite acceptable but efforts should be made to keep track of which animals spawned as male and/or female. When putting animals together for a spawning there should be individuals from a variety of sources. The objective is to end up with as much mixing as possible.

As experience develops in different places some stocks may be identified as being better performing in a farm situation or for certain traits. As this information becomes available it will become important in decisions for importing strains for farming. Whether good performance of a strain in one place will mean good performance in another will have to be determined. The geneticist calls this **genotype-environment interaction**, and we know nothing about its importance in giant clams.

When importing to re-establish stocks of giant clams concern is needed for the adaptations of the animals to local environments. If the stock is to establish a self-recruiting population it will have to be fit in the local environment. As in the farming situation, one should use residual local stock if they are available and incorporate them. The same concern about basing the stock on very few individuals applies. Probably the best sources

of stock would be those within the regions shown to be genetically similar by the population genetics studies. The study of the ecological parameters would add to this in determining similarity of source and local environments.

Importations of stock should be controlled by concerns about nongenetic effects such as the possible introduction of disease and pests. There are international protocols for the introduction and transfer of species which should be used. It would be worthwhile examining these protocols to see how they can be made specific to giant clams. One of the difficulties of the quarantine procedures and other controls used is the real chance of reducing the amount of genetic variation transferred. The pathologists would like to see as few animals and as few shipments as possible. The geneticist would like to see many animals because it is primarily in transferring animals that genetic diversity is transferred.

One way of transferring genetic diversity that may be easier with respect to disease and pest transfer is to use cryopreserved sperm. If sperm from many males can be collected and transferred it would help in increasing the genetic diversity. Cryopreserved sperm is not a panacea because it is the source of only half of an individual's genotype. It is still necessary to have many individuals as females.

When starting with an undomesticated population and introducing it to a farm environment, selective mortalities will occur (natural selection), and individuals will be selected as broodstock based on performance (artificial selection). In other words, genetic change, hopefully for the better, is bound to occur and it will start immediately. Thus, it is wise to make a good start in the first generations in obtaining sufficient numbers of parents. If wild stock is introduced several generations later to inject genetic variability, undesirable genes will also be injected, ones which had been carefully selected out. There are reports of renewed vigor resulting from outcrossing cultured fish stocks to wild stock but the explanation probably lies in the fact that the cultivated stock had become inbred, so instead of being improved over the wild stock it was actually deteriorating. The best approach is to start right and maintain good control over the stock. If it seems necessary to introduce new stock (wild or otherwise) they can be developed as separate lines and crossbred to the old stock when it is certain that overall improvement will result.

Broodstock Management

For discussion purposes this treatment of broodstock management has excluded selection procedures. In practice the two must be considered together. However, here we will discuss those aspects of propagating and rearing the broodstock which pertain to:

a) maintaining the broodstock without loss of genetic variation and avoiding the accumulation of inbreeding
b) rearing the broodstock while maintaining the identity of progeny groups and providing an evaluation of their performance.

Inbreeding of broodstock is to be avoided although there is only limited evidence as to the specific effect of inbreeding in bivalves. The evidence we do have and conventional breeding experience suggest a significant inbreeding depression (loss of vigor and performance) is likely. Certainly there will be a loss of genetic variation and thus loss of potential for response to selection. Whether there is an intensive selection program at the hatchery or not, propagation of lines should be done to minimize the accumulation of inbreeding.

Inbreeding will increase as the sex ratio deviates from 1:1. (Think of the clams as "functioning" as separate sexes in a genetic sense.) Taken to the extreme though the broodstock may consist of hundreds of parents, if only one individual were used to contribute sperm, all the offspring would be half sibs. It is recommended that an equal sex ratio be used to advance each generation. In addition there should be 50 pairs of adults each generation for each stock or line. This would result in an inbreeding rate of 0.5% per generation and a total accumulation of inbreeding after 5, 10 and 20 generations of 1%, 3% and 5%, respectively. At a moderately low level of inbreeding, natural and artificial selection should counteract the negative effects of inbreeding.

It seems unlikely that each giant clam hatchery will be able to maintain 50 families every generation. There are ways of achieving

the desired goal of minimizing inbreeding and maintaining genetic diversity but it will require coordinated effort from several groups. Efforts such as saving separately a few hundred offspring from a partial spawning, which would not make a large enough batch for commercial spat production, will help in achieving the genetic goals. The point is that at some time some of the offspring of each of 50 pairs should be set aside to develop into broodstock. This can be done at any stage and from spawnings that occur at different times and places. One simply must be able to identify the line and generation of the individuals the next time broodstock is to be set aside.

If progeny are set aside for broodstock from production at different times and different places care should be taken not to inadvertently eliminate some groups because of selection for size. Groups handled in different ways or at different times are very likely to have different mean sizes. Most of the differences will be nongenetic, hence, the individuals should not be culled merely on the basis of size relative to the overall mean. Consideration should be given to the individual size relative to the group (e.g., family) mean size. Otherwise, the contribution of some groups of parents will be eliminated without proper evaluation.

It is inevitable that at some time the number of parents will be reduced either through failure of maturation, mortalities or accident. The reduction of parents in one generation will create a bottleneck in the maintenance of genetic variation.

More control can be exercised and thus less inbreeding will occur if separate lines are maintained. The maximum control is obtained by maintaining separate families identified through maturity. In this way crosses can be made between families in such a way that the nearest common ancestor is many generations back in the pedigree. If the founding stock was derived from a small number of parents (10-20) it is strongly recommended that separate families be maintained at least in the first generation. Thereafter a number of pooled lines can be formed by careful crossing of the original families.

Maintaining several different lines and using a special crossing scheme can be more effective in reducing inbreeding than maintaining one large line with the same number of parents. An effective crossing scheme has been worked out with fish called rotational line crossing. This involves crossing the females of one line with the males of another (using three or more lines) in a rotating manner each generation. With as few as three lines a significantly more effective program can be maintained.

The broodstock may not need to be propagated each time it is spawned if multiple spawnings are planned for some individuals. This will depend on the facilities and the hatchery management. The broodstock propagation should be planned in conjunction with spat production but it is a separate activity. Hopefully there will be a selection program to be included as well, but this is not considered here.

In rearing the future broodstock one must know how many of each family or line will be needed at maturity. Then using the expected survival at each stage it is possible to calculate the number of larvae and spat that are needed. Of course, the next-to-worst scenario should be assumed. Low but reasonable estimates of survival should be used. (The worst scenario is 100% mortality in which case it does not matter how many are saved!) With realistic estimates the cost of maintaining the broodstock can be kept at a minimum.

When different families or lines are maintained it is necessary to know the family or line identity of each individual at the time of spawning. At present there is no convenient way to tag larvae or small spat. Thus, it is necessary to maintain eggs and small spat in separate containers until they are large enough to tag. Several techniques have been developed for tagging clams. "Genetically" tagging clams by using electrophoretically detectable gene markers or DNA fingerprints may be feasible.

It is necessary to have some evaluation of group and individual performance on which to base broodstock selection. What traits are important will be decided in designing the selection program.

There is reason to expect significant variation in the performance of different groups as a result of the different tanks or trays they are raised in. This will become more of a problem when the groups are separated by space or time. These differences may be due to random effects of variation in water flow, light

or nutrient or systematic effects like different management schemes. If families or lines are held in separate tanks then these tank effects will become inseparable from the genetic effects when evaluating performance.

Whether this is a serious problem depends in part on the traits being selected. If selection is primarily based on later performance of individuals, then environmental influence at early stages is probably not serious since there is probably a low correlation between early performance and later performance of traits like growth rate. Obviously the magnitude of the correlation depends on how much time and growth has elapsed between "early" and "late".

Even if control of spawning or limited hatchery facilities limit the control of spawnings to small groups of animals that are mass spawned (no control over individual fertilizations), control of the broodstock is not very difficult. If two individuals are known to come from different spawnings in the previous generation they will not be related. An example will best illustrate the principle. In the following, clams are considered to come from two different sources, A and B. In the table the number of males and females of A and B are shown for each spawning. Over 3 years there can be a reasonably large number of animals from these two sources spawned. The years 1992 to 1994 are considered the first generation of this stock.

	Females	Males	Identification
1992			
	2A	5B	92-1
	3B	4A	92-2
	3A	5B	92-3
	1B	3A	92-4
Total	9	17	
1993			
	3B	6A	93-1
	4A	7B	93-2
	2B	4A	93-3
	5A	6B	93-4
Total	14	23	
1994			
	3A	6B	94-1
	4B	7A	94-2
	5A	7B	94-3
	5B	8A	94-4
Total	17	28	

If these are *T. gigas* they will not be female mature until 1999. At that time the females can be taken from the 1992 clams and males from 1993 (or 1994). By making the following crosses no inbreeding occurs:

	Males	Females	Identification
1999			
	5 92-1	8 93-1	99-1
	5 92-2	10 93-2	99-2
	5 92-3	9 93-3	99-3
	5 92-4	15 93-4	99-4

In such a program it would be important to use animals as only one sex. It is assumed that control of spawning will increase over time. (For the same reason this example shows an increase in the number of animals spawning over time.)

Conclusion

No specific plan has been laid down here. The intention was to discuss some of the underlying principles so that discussion with people involved in production could identify the biological and technical constraints and opportunities. There are many variables to consider and the best approach for any particular case will be a unique set of compromises.

One cannot expect that in all hatcheries a major effort can be made. Nevertheless, some effort should be made in all cases. The key word in the management of broodstock is control. Control in establishing the broodstock will insure that the foundation is present for a long-term program. Control of broodstock maintenance will insure that genetic variation is not lost inadvertently and that inbreeding will be avoided.

Discussion

PULLIN: On the point whether to use local stock or import stock, I think this workshop should come up with some strong guidelines. There has been a lot of misdirected work on this. Often some consultants, some foreign institutions and maybe some commercial institutions say what you need is not only our advice but our animals, because your local stock is not worth anything. This has happened in Malawi for example, where it led to the introduction of the carp. It has created a mess, and they're now trying to eradicate some species. It has also happened in West Africa in

French-funded work with an exotic lagoon species, no proper evaluation of the local species and stock was made. The default option is not to introduce, but to really assess and this is rarely done. I think we need to send a strong signal on this.

In terms of gene banking and cryopreservation, the fact that one can only store sperm is not a problem for giant clams, as they are hermaphrodites.

NEWKIRK: It's a question of sampling, but you've still only got half the genotype.

BRALEY: Say we have 3 different populations and we can get 50 pairs from the Solomons, the GBR and say the Marshalls - do we bring them all together in one spot or what are you recommending?

PULLIN: You should avoid the kind of institutional and political pressure that goes with financial support. If you bring in animals it should be a reasoned decision. Some framework is needed for making these decisions.

CALUMPONG: We have only very small stocks of *T. gigas* in the Philippines, and we have been trying to spawn them unsuccessfully. So the reality is that we have to use stock from the Solomons and from the GBR, and maybe we can get some Philippine sperm to mix in.

NEWKIRK: We don't know how much value to put on the sources of differences in the stock.

GOMEZ: Although the Philippine situation does present problems it also offers some unique opportunities. At UPMSI we have five lines of *T. gigas*, two from the GBR and three from the Solomons. So we have five lineages or populations. The sixth one is the lone Philippines *T. gigas*. One of our main interests is to try and get *T. gigas* off the ground in the Philippines from these very small numbers, but how we are going to do that is an interesting problem.

Unlike tilapia, the giant clam, at least *T. gigas*, does not spawn easily. One of the more manageable species may be *T. crocea*. In my experience it is easier to spawn, and it is fairly widespread. There is some interest in that species both in the foreign trade and for food.

NEWKIRK: An animal breeder would not consider the five lines you've talked about as lines. It is essential to track your batches, so that in 10 years you can go back to your records and say that animal came from a spawning that involved these animals, and not those. You may not be able to tell exactly which its parents were, but that is the level of pedigree we can deal with on a realistic basis.

PULLIN: Why don't you throw out the concept of 50 pairs for captive breeding ? There is the possibility of having lines which are self-fertilized, so if you have a very small population, even 5-8, you can get these animals to fertilize themselves and then have crossing programs.

NEWKIRK: I don't react very warmly to using self-fertilization. In cases where it is effective in plants or animals they are big programs, they have many many inbred lines, probably many more than 50. I think the outcrossed option is better.

PULLIN: I agree, but where you cannot get 50 pairs by a fault of history, would not the inbred lines be the best option ?

BENZIE: I can't react to that very warmly either because as Gary has said very very high numbers are needed.

Conservation of Wild Stocks: Policies for the Preservation of Biodiversity*

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BENZIE, J.A.H. 1993. Conservation of wild stocks: policies for the preservation of biodiversity, p. 13-16. *In* P. Munro (ed.) Genetic aspect of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction

Giant clams have become rare or extinct over much of their range because of overexploitation (Copland and Lucas 1988). Techniques recently developed to farm giant clams now provide a means of restocking depleted populations (Braley 1989). Early work implied that giant clam populations were not genetically structured (Campbell et al. 1975).

*Contribution no. 819 from the Australian Institute of Marine Science.

Under such circumstances transfers of live material throughout the Pacific from any source might be considered useful enhancements of local populations. However, recent studies have provided evidence of significant genetic structuring of giant clam populations in the Pacific (Benzie and Williams 1992; Macaranas et al. 1992). Strategies for the protection of the giant clam need to take account of this genetic diversity. New approaches will need to be developed if the transfer of cultured material for restocking does not serve to destroy the resources they aim to enhance.

Conservation of Biodiversity

There has been considerable attention paid recently to mechanisms for maintaining biodiversity (Soule and Wilcox 1980; Soule 1986). These have focused on the one hand on the design of nature reserves, the concept of minimum population sizes and the need for connectivity between populations. On the other hand, great effort has been expended in developing breeding programs for small populations of endangered species in zoos, where the risk of inbreeding is very high. Both approaches have relevance to giant clams, but there are additional issues for these species concerning restocking, and the potential development and spread of domesticated strains, that are not addressed in standard conservation biology texts. Similarly, the value of wild genetic resources to industry and to agriculture are widely recognized (Oldfield 1989), and strategies for the documentation, collection and maintenance of such resources have been discussed by Brown et al. (1989).

Reserves

Fundamental to the protection of biodiversity is the establishment of a network of reserves each of sufficient size that the populations are self sustaining. The network of reserves should encompass the bulk of the biodiversity which it is sought to protect. Strategically placed such reserves can, at least in theory, act as a source of recruits for areas that are exploited. The presence of genetically different giant clam populations in the Great Barrier Reef, the Solomon Islands and different parts of Micronesia implies reserves for clams be situated in each of these areas.

Reserves targeted at preserving general marine faunas should serve to protect the giant clams in those habitats. The extent to which such reserves can act as sources of recruits to other sites depends very much on a number of factors such as their hydrodynamic relationships and the density of clam populations within the reserves. The processes of natural recruitment of clams can be assisted artificially by grouping animals together so they are more likely to fertilize each other, although the dangers of disease and predation are also increased by this method. It is stressed that this approach is probably the cheapest method of maintaining diversity, and the source of future strains for aquaculture.

Gene Banks, Cryopreservation

Cryopreservation of eggs, sperm, or germplasm, and the storage of cell cultures or seeds represents an alternative method of maintaining genetic variants. However, the maintenance of the collections is expensive, the collections are necessarily limited, and as a result ownership and access to the material can present problems (see Brown et al. 1989). Techniques for cryopreservation and cell culture of giant clams have yet to be developed for clams, and may well be of use in maintaining gene banks of cultured strains, and a reference collection of wild ones. However, in the context of this paper, the use of these techniques implies a failure to achieve this primary aim of the preservation of wild stocks.

Captive Breeding

The considerable work recently with zoo populations has shown that much can be done to prevent the loss of genetic variation among very small populations by the use of carefully designed mating schemes, often achieved through artificial inseminations (Soule 1986). Given the capability to obtain gametes with relative ease from giant clams these methods may be of use in trying to build up populations from small numbers of survivors in a particular region. The approach is dependent upon careful monitoring of the matings achieved, and can be

enhanced considerably by use of sensitive DNA markers. However, the clear consensus is that the method is a last resort, and for organisms that may exist nowhere else. It may be useful to consider in conjunction with small reserves in places where only a few individuals remain, e.g., *T. gigas* in the Philippines.

Restocking

Rather than leave natural populations to self-recruit, animals can be introduced from elsewhere to enhance populations at particular sites. In areas where animals have become so rare that they are unlikely to breed, or where they have become extinct, this is the only approach available. The existence of genetic differences among giant clam populations means that care in planning restocking programs is needed if the process is not to eliminate local diversity. If significant local populations occur the introductions of material from elsewhere should not be encouraged. On present evidence, one might suggest that *T. gigas* from Australia or the Solomon Islands not be introduced to Micronesia, as significant stocks of a more appropriate genetic constitution are available in the Marshall Islands. On the other hand, introductions to the Philippines would only involve populations between which there appears to be reasonable genetic exchange already.

Most restocking involves the use of cultured animals because of the logistic and economic advantages of introducing large numbers of small animals rather than large adults. The solution to enhancing genetically different populations is not simply to apply current culture techniques to broodstock obtained locally. Mass producing animals from few adults, as happens at present, serves to reduce genetic diversity, and creates major shifts in the gene frequencies of the cultured populations relative to their wild parents (see Benzie, this vol., p. 1). A revision of hatchery techniques will be required to produce genetically diverse batches. Restocking programs may require several introductions over time, and include the progeny from many matings in order to produce populations whose gene frequencies approach those of natural local stocks. Basic approaches from which specific strategies can be developed are available from standard quantitative genetic work, and extensions of this from the zoo breeding programs (Soule 1986). There have been no precedents for this approach to restocking, and monitoring the effects of different management strategies will be important.

Domesticated Strains

Where the aim is to restock reefs to maintain local genetic diversity, or indeed to ensure that animals reintroduced to reefs from which they have become extinct have a sound, diverse genetic base, there is no conflict in the goals to be achieved. However, the development of domesticated strains for more efficient aquaculture and improved food production demands a different approach. Should introductions of a domesticated strain be considered, and the introductions are to a region which has its own locally diverse populations, there is a direct conflict. Oldfield (1989) has documented the effects of domesticated strains on wild populations, and on species which are close relatives, with which they have interbred. Brown et al. (1989) detail the effort and cost of obtaining genetic material for agriculture from rare wild stocks after such loss. There are advantages, such as enhanced growth rates, to developing animals for food production that cannot breed, so that they will not endanger local stocks. A relatively small broodstock population could be managed in a way that would minimize the likelihood of their breeding with wild stock.

Conclusion

At present there are no genetically improved domesticated strains of clams available. Until then, one might approach transfers and introductions from the perspective of restocking. It is clear that transfers between genetically distinct groups should not be made if the genetic diversity of wild stocks is to be maintained. Tranfers within groups should use techniques that do not reduce variation in introduced stocks, and approximate the genetic constitution of the local stock. The future challenge will be how to deal with the spread of a farm animal, and whether a key goal in doing so should be to develop one whose production components cannot breed. The proposal is not simply one of conservation but

of the cheapest and most effective way of maintaining resources upon which future biological developments will depend.

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Discussion

GOMEZ: How far apart must your islands be geographically to manage introductions on a genetically sound basis ? Here in the Philippines we have some 7000 islands. How do we manage this kind of situation?

BENZIE: It's not a question of geographical separation necessarily. If you have the last remaining clam in an area and you bring in clams from a nearby place you are not doing any harm. There's a spectrum from a zoo type situation to the one in the GBR, the Solomons or the Marshall Islands, where there are large natural viable populations that can be used as sources for local spread. Here you can have reserves where the local populations are protected from exploitation and from aquaculture. So I can't give a precise answer to your question.

General Discussion 1

PULLIN: Any intervention that we make due to development objectives will have environmental consequences and sometimes a genetic impact. The question is how much does that matter on balance? These extremely small stocks, almost relic stocks, that you talk about are like a terminally ill patient. It would be perhaps rather silly to hold off from potentially beneficial intervention to maintain their genetic integrity. We should assess what the genetic impact may be and then make a decision. Against that, giant clams like fish, have much larger families than pandas, and therefore the prospect for a captive support breeding population swamping a resident population is there, and the genetic impact has to be considered. This is what some of the IUCN captive breeding groups are looking at, if they're able to release birds or mammals which are equal to or better than the surviving population, they're going to have a huge genetic impact. We could do that in one or two generations with aquatic organisms. So we should assess the genetic impact before intervening.

J. MUNRO: In the case of giant clams natural rates of recruitment are remarkably low, and by releasing hatchery-reared batches in a protected area the natural stocks will certainly be overwhelmed.

MACARANAS: Can we take the GBR situation as a model for the Philippines, where the GBR results show that within a certain distance there were no significant differences in the stocks? Can we superimpose these results on the Philippine situation, and assume there will be no differences within the same geographical distance?

BENZIE: Here I can give a very definite answer. That is No. The GBR is a very special situation, and it has unique features, characteristic current flows and highly interconnected areas.

NEWKIRK: On the east coast of the US the oyster stocks are very similar in terms of some allele frequencies, yet over a 500 km range they have a very different physiological adaptation to times of spawning, etc. You cannot see the kinds of differences you're talking about here using these techniques of measuring population differences. So while it would be nice to measure the genetic impact it's not practical.

Maybe we shouldn't even look at the clams that are left, maybe they're very unique. Why are they left? Maybe because they're slow growers.

BENZIE: In terms of a sample of the genome we're looking at, it's certainly very small.

GOMEZ: Last year I got some very beautiful *T. crocea* from the Pacific side of Luzon, some 40 animals with very beautiful colours, blues and greens, etc. We also brought some from the Cebu area where they are heavily fished - they are all brown. These are the survivors of a population that is well camouflaged and not so easy to find. Human pressure is exerting a selection on the animals.

J. MUNRO: All fishing creates selective pressure.

GERVIS: We have been ignoring the zooxanthellae.

BRALEY: The iridiocytes in the tissue of the clam confer its color. But where there are high nutrient supplies the number of zooxanthellae increases and the color of the clam changes.

HESLINGA: There is a correlation with the depth of the water. The zooxanthellae have more pigment in deep water.

J. MUNRO: The zooxanthellae are certainly very important from the genetics point of view.

BENZIE: The enzymes systems we looked at in the population studies were without doubt specific to clam tissue, not zooxanthellae.

NEWKIRK: It might be more important to look at the genetics of the zooxanthellae. Are they species specific?

J. MUNRO: No. We have given zooxanthellae from *Hippopus hippopus* to *T. gigas* and various other combinations and they have grown well with zooxanthellae from other species.

P. MUNRO: The molecular biologists who are working on this have shown that various strains of zooxanthellae occur in widely differing host species. Although all the host species within a particular area will take up the same strain of zooxanthellae, showing there is selection by the host, the same strain may be found in unrelated host species.

BENZIE: Any interaction between zooxanthellae genotype and the clam genotype and growth rate would be quite important.

J. MUNRO: Our group has shown that the zooxanthellae can be taken up by the clams up to 38 days of age; we don't know about beyond that. This means that the juveniles can be shipped without introducing zooxanthellae, which are an added source of infection. We also know that larvae grow better with zooxanthellae taken from fast-growing clams than with slow-growing clams from the same cohort. We would like to know if there is turnover of zooxanthellae in the clams at later stages.

Strategies for Re-establishment of Wild Giant Clam Stocks*

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MUNRO, J.L. 1993. Strategies for re-establishment of wild giant clam stocks, p. 17-21. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction

Stocks of giant clams have become severely diminished in almost all areas of the tropical Indo-Pacific, mostly as a result of intensive harvesting for subsistence purposes or, in the case of remote areas, as a result of intensive commercial gathering for the Taiwanese market (Hester and Jones 1974; Munro 1989). Additionally, it appears that there might have been a natural contraction of the range of *Hippopus hippopus* and *Tridacna gigas* as a result of climatological changes over many centuries.

Natural recruitment to stocks appears to be low and episodic (Hester and Jones 1974; Braley, 1988; Adams et al. 1988; Pearson and Munro 1991), although McMichael (1975) observed fairly regular annual recruitment of *T. maxima*, equal to about 10% of the stock, at a study site on the Great Barrier Reef.

*ICLARM Contribution No. 920.

Clearly, recruitment rates can be expected to be related to the numbers of fertilized eggs released into the water column and a diminished stock will be expected to yield an equally diminished cohort of recruits. There is no evidence that the prospects of survival of recruits are in any way enhanced by decreases in the abundance of adult stocks, although this could be the case in the dense stocks of *T. maxima* in some atoll lagoons in French Polynesia (Richard 1978).

A feature of the biology of tridacnids which will limit recruitment in depleted stocks is that eggs have an associated chemical substance (Munro et al. 1982) which induces sperm production in response to its detection by another clam. If stocks are drastically depleted, there is an excellent chance of a plume of unfertilized eggs never encountering a second clam, with consequent failure of the entire spawning. The length of time that an unfertilized egg remains viable is not precisely known, but experience in hatcheries suggests that it is less than 24 hours and that immediate fertilization is optimal.

Additionally, experience gained in ocean nurseries suggests that survival is positively correlated with growth rates. Presumably this is even more pronounced in unprotected wild juveniles where the coefficient of mortality can be expected to be directly related to the length of time that a clam remains below a given size. This suggests that natural stocks of giant clams have already been intensively selected for rapid growth and resistance to parasites, diseases or predators and can be expected to grow faster than cultivated stocks. The reason for the enormous variability in growth rates in the progeny of wild-caught broodstock is not yet understood by geneticists (Thiriot-Quiévreux et al. 1991).

Strategies for Re-establishing Wild Stocks

In countries where giant clam stocks have been drastically depleted, two basic situations can exist: either wild broodstock are extinct or nearly so, or sufficient numbers can be located by intensive searching to produce a modest aggregation of local stock. Clearly in the latter case it is imperative to conserve and propagate the remaining genotypes represented by the stock.

If the local stock is extinct, it becomes necessary to import either spat or adult broodstock. The second option carries risks associated with any ocean-to-ocean transfer. Importation of early spat (14-28 days) has much diminished risks, particularly if zooxanthellae can be added at the receiving end and a major source of possible contamination of cultures thus removed. It has recently been shown that it is possible to maintain larval cultures without zooxanthellae, but fed on artificial microfeeds, up to 38 days (Molea 1992).

It would appear to be self-evident that for the purpose of recreating a stock, that the largest genetic diversity should be sought. That is, successive cohorts imported from a given source should be relatively small and derived from different parents on each occasion. A question which should be addressed at this workshop is whether or not spat should be imported from a single location, based on desirable characteristics (in addition to availability), or whether the greatest possible genetic mix should be sought in order to maximize heterozygosity and diversity and a "new" stock thus created.

The key difference between restocking programs and farming systems is that the farmed stock is, or should be, destined for harvest before reaching sexual maturity. This would ensure that wild stocks are not unnecessarily contaminated by domestic stocks of low heterozygosity. This is a factor that should be considered in economic analyses. The onset of female maturity would appear to be the critical point because the fertilization of a batch of eggs spawned by a wildstock clam by a mass release of sperm from a cultured stock would have no unusual genetic consequences, whereas the mass release of eggs by cultured stock and resultant release of sperm from the same stock would possibly result in the dispersal of enormous numbers of larvae of very limited heterozygosity.

There are few published data on size or age at maturity of tridacnids. Nash et al. (1988) reported that *T. gigas* attained male phase maturity at 25-35 cm SL but gave no information on the smallest female phase clams encountered. At the Coastal Aquaculture Centre in the Solomon Islands, the smallest *T. gigas* which has produced eggs to date was 38

cm shell length (SL). However, as shown in Table 1, most *T. gigas* do not produce eggs until they are over 55 cm SL.

If restocking programs are to be undertaken, based on hatchery-reared stocks, it may be that the stock should not be culled or selected in any way, in order to maintain the greatest diversity. Clearly, all hatchery and nursery procedures are selective to some degree and this cannot be avoided, but at least should be minimized.

Restocking Programs and Marine Protected Areas

The "release" or distribution of hatchery-reared juveniles in heavily exploited areas is a form of fishery enhancement or supplemental recruitment and if harvesting pressure is excessive, will merely raise the total catch by the ratio that the hatchery-reared recruits represent relative to the total number of natural recruits.

The chances of any supplemental recruit reaching sexual maturity in an already-depleted area are minimal and it is therefore likely that the creation of marine protected areas (MPAs) is an essential adjunct to re-establishment of stocks. The location of MPAs is important (Anon. 1990; Polunin 1990) and it can be deduced (Williams et al. 1984; Wolanski and King 1990) that complex reef systems in areas of modest currents will have the greatest chance of retaining larvae, whereas fringing reefs on a linear coastline would have the least.

The concept of MPAs as reservoirs of breeding stock is well established (Salm and Clark 1984). The use of "clam circles" to promote natural restocking of adjacent reefs has been advocated (Chesher 1991). Given that clams tend to thrive best in areas with relatively strong currents, arranging the clams in a single circle would seem likely to ensure that close to 50% of all batches of eggs will be wafted away from the circle and will not be fertilized. An aggregation of the same number of clams, either randomly or systematically distributed within a circular patch of reef would have a much lower incidence of total loss of reproductive products.

A negative aspect of the entire concept of re-establishment of stocks simply by the aggregation of broodstock in marine protected areas is the extraordinarily low natural rate of survival of larvae and juveniles. Although we lack detailed estimates of fecundity for the larger species, Jameson (1976) described the fecundity:shell length of *Tridacna maxima* as $F = 0.00743\ L^{4.03}$. A 200-mm *Tridacna maxima* would therefore produce about 13 million eggs. We also know from hatchery experience (Table 1) that the release of 40-240 million eggs by a single *Tridacna gigas* is not uncommon and that an individual can produce these quantities of eggs several times per year.

The average size of mature *T. gigas* at Michaelmas Reef, Great Barrier Reef, was about 78 cm in 1978 (Pearson and Munro 1991; Table 5) implying an average reproductive life of about 20 years and the production of about 6×10^9 eggs (20 years x 3 spawnings x 100 million eggs) in a life-time, of which only one need survive to maturity to replenish a stable population. The survival rate at Michaelmas Reef was such that about 170 two-cm recruits were needed to provide one 74-cm adult; but only fifteen 14-cm clams would be needed for the same purpose. In ocean nurseries and exclosures in the Solomon Islands around 30% survival of 2-cm clams to 14 cm is currently achieved, whereas at Michaelmas Reef only 9% appeared to survive.

The conclusion is that clams stocked into MPAs should be held in protective exclosures for as long as is feasible but, given that maturity

Table 1. Size at female maturity of *Tridacna gigas* at the Coastal Aquaculture Centre, Solomon Islands.

Size group (cm)	# tested	# producing eggs	Maximum # of eggs produced
35.1-40	1	1	"very few"
40.1-50	0		
50.1-55	7	1	46
55.1-60	9	2	35
60.1-65	16	10	70
65.1-70	16	15	25
70.1-75	10	7	240
75.1-80	6	5	200
80.1-85	1	1	45
85.1-90	2	2	

of unselected stocks at 50 cm is only attained at an age of 6-10 years (is maturity age or size related?) it will take up to 10 years before any larvae are added to those of adjacent wild stocks and perhaps 20 years before the surviving broodstock have a major impact, because of the low fecundity of the young broodstock.

Pearson and Munro (1991) estimated that 7,298 2-cm recruits would have provided the 287 72-76 cm *T. gigas* observed on a 2.7-ha plot at Michaelmas Reef and if the stock were stable this number would be needed every year. In fact, numbers observed were only a very small fraction of this (Pearson and Munro 1991; Table 5), indicating that conditions for settlement and survival of recruits to the reef had changed over a period of about 20 years.

Given that giant clam stocks on the Great Barrier Reef are wholly protected and in a near pristine state, the indications are that natural recruitment is limited by episodic events, is erratic and therefore cannot be relied upon to replenish exploited reef areas unless there are very large stocks of broodstock in adjacent MPAs. However, it is also likely that the incidence of larger predators will be less in exploited areas than on the Great Barrier Reef.

Conclusion

It is technically feasible to re-establish stocks in MPAs, particularly if ocean nurseries and exclosures are used to protect juvenile clams to the largest possible size.

Natural recruitment rates of giant clams appear to be extraordinarily low, despite the prodigious fecundity. Very large stocks will be required in MPAs in order to have a positive impact on recruitment to adjacent exploited areas.

If farming giant clams is economically viable, stocking unprotected areas with hatchery-reared recruits would be a poor substitute, due to the low recruitment rates to be expected.

Adverse genetic effects can be avoided by a policy of harvesting all farmed stocks before female maturity is attained; except for individuals selected for future breeding programs.

All selective mechanisms should be avoided to the greatest possible degree when giant clams are produced for the re-establishment of stocks.

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Discussion

BRALEY: I took gonad biopsy samples on a regular basis of discrete populations of clams every month for two years. There was no spawning in the first summer at all, the first spawning season. In the second year almost all the clams spawned. So unless the conditions are just right they may not spawn every year in the wild.

J. MUNRO: In the central tropics our experience is that *T. gigas* is more ready to spawn. Almost all our broodstock have produced eggs over the five years we have been going, and some spawn more than once a year. We rotate our broodstock between the sea and the tanks, so we don't know exactly how often they spawn.

BENZIE: Firstly, if you have very low standing stocks will they ever become self-sustaining? Secondly, the time scales involved in relation to the need for genetic diversity and the constraints of the hatcheries in producing lots of batches mean that restocking may take place over a number of years.

J. MUNRO: Certainly restocking doesn't all have to be done at once. Gary was talking about 50 pairs of animals - that's only 7 families per year and well within our present capacity.

HESLINGA: We are dealing with two if not three issues here. 1. Preserving biodiversity 2. Farming for whatever reason 3. Stocking. We have to find where these three approaches intersect in order to make recommendations to the farmers. I agree with John that restocking is not going to happen by putting baby clams on the reef. This may be an idea which no longer has any advocates.

J. MUNRO: If you want to re-establish wild stocks you are going to have to go into farming mode within your protected area and rear heaps of diverse families up to maturity. So your restocking strategy is a blend of farming and reseeding.

ALCAZAR: Local farmers are not concerned with restocking the reef. What they are after is income. So they collect and collect and that is the problem here in the Philippines.

BENZIE: You have to consider what the pressures are to have stocks that are becoming extinct regenerate. If the pressure is simply to have food for the local people, perhaps the best technology is just to have farms. Fish restocking excercises in the past have not been well monitored to my knowledge. We are seeing a mix of end points here. We should look at each situation and see what the goals are, and whether differing goals affect each other and try to assess the impact.

Giant Clams, Genetics and Hatchery Procedure*

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Introduction

Genetics has not been a major topic of giant clam research to date as different groups have been establishing what constitutes the most favorable environment in which to raise these animals. All giant clam breeding centers have unintentionally or intentionally practised some form of selection both with regard to the giant clam itself and its symbionts. The Micronesian Mariculture Demonstration

*ICLARM Contribution No. 921.

Center has been breeding from their own broodstock since 1984 (Heslinga et al. 1988). It has been shown that hatchery-reared clams have a reduced genetic variation and skewed gene frequencies when compared to wild stocks (Benzie and Williams, unpubl. data). It has also been shown that inoculation of larvae with different strains of their symbiont influences their growth rate (Molea 1992), so that there are two genetic systems to consider in giant clams.

Giant clams pose special practical problems in terms of genetic management due to the size of the broodstock (especially *T. gigas*), and their symbiotic relationship with zooxanthellae. Hatchery protocols vary throughout the region. This paper discusses some of the qualities of giant clams (especially the larger species *T. gigas* and *T. derasa*), and specific hatchery procedures used at the CAC that have implications for genetic management of giant clams.

Parent Stock

T. gigas parent stock have been gathered from five main areas of the Solomon Islands covering a 400-mile range. This stock has been shown by Benzie and Williams (unpubl. data) to have a heterozygosity of 0.297 which is higher than the heterozygosity of other populations of giant clams studied in the Pacific. The total number of *T. gigas* broodstock at the CAC at the time of this writing is 76 and these come from six areas; 14, 7, 11, 16, 25, and 1 clam, respectively, from each area. There are smaller collections of parent stock of *H. hippopus, T. maxima, T. derasa* and *T. crocea*. For the production of more than 500,000 10-mm juveniles a year, 200 broodstock from a wide geographical base is recommended.

Parent stocks have not been evaluated for different phenotypic qualities such as weight:length ratio, meat weight:shell weight ratio and shell structure. These factors can give some indication of growth rate but it is not yet possible to tell in the field whether poor growth patterns are genetically or environmentally caused.

The maximum size of *T. gigas* parent stock gathered has been 90-cm shell length; such a clam weighs more than 120 kg and is the maximum size that can be man-handled across reef flats without machinery. The largest clams do not respond well to being transported out of water as their meat can collapse inwards killing the clam (Govan 1988). Therefore a certain amount of selection has already taken place while collecting broodstock.

Large parent stock are either double-tagged using animal eartags or number-punched aluminium tags riveted into the shell. For the smaller species, glue-on shellfish tags, metal tags or dymo tape embedded in epoxy glues are used.

Aspects of Broodstock Management

Broodstock Size

Unlike oysters, manila clams, mussels, scallop and other bivalves, either the facilities needed for holding broodstock giant clams (*T. gigas* and *T. derasa*) have to be very extensive or the number of broodstock to be spawned at one time must be limited (5-30 individuals). This does not present a problem when selective spawnings are required, but narrows the potential number of parents contributing genotypes at a spawning.

Size and fecundity appear to be positively correlated. The most eggs obtained from a single broodstock at the CAC has been 240×10^6 from a 77-cm clam.

Hermaphroditism

The hermaphrodite nature of the giant clam presents advantages and disadvantages to the culturist in terms of genetic management. The obvious advantages are that any given clam can produce either sperm or eggs, and sexing is not a problem. The major disadvantage is that sperm release nearly always occurs prior to egg release, and self-fertilization is hard to control. If sperm release occurs from more than one clam it is hard to identify the parents. Current practice at spawnings involves flushing away sperm from the tanks after retaining some for subsequent fertilization of the eggs. For improved control the clams need to be spawned in separate containers. This is relatively easy for the smaller species.

Fecundity

T. gigas and *T. derasa* are fecund animals with the largest clams releasing up to 500 million eggs in a single spawning. The complete set of hatchery tanks (315 m^2) at the CAC can

be filled from a spawning of 100 x 10^6 eggs assuming a 4.725% survival rate to 28 days old and a stocking density of 1.5 juveniles cm^{-2}. It is therefore unnecessary to obtain eggs from more than one clam at a spawning and mass spawnings nearly always result in discard. Eggs are obtained from the smaller species by placing the spawning clam in an individual container and either letting it become spent completely in the same container or, if the eggs are too dense, transferring it into another container during the spawning. Eggs from *T. gigas* are caught in plastic bags as they are released from the clam and transferred directly to the hatchery tanks.

The fecundity of *T. gigas* means that a complete hatchery system can be occupied by progeny from two parents for 3-6 months; this restricts the number of cohorts produced. If the progeny are then shipped together to growout areas, a lack of genetic diversity will arise in the growout areas.

Efforts should therefore be made to increase the throughput of clams in the hatchery and to ensure a wide but mixed transfer of clams to growout areas. Using floating ocean nurseries clams can be transferred to sea from the hatchery tanks at 3-4 months old which means only a 2-3 month period in settlement tanks, as the first month is spent in larval tanks. In theory, 4-6 cohorts could be put through the hatchery system per year if broodstock conditioning could be perfected and spawnings assured. Each cohort could be graded heavily by day 28 to vacate tank space, but the effectiveness of grading prior to the stocking of settlement tanks is not proven yet. The smaller species are less fecund.

Cryopreservation

The natural fecundity of giant clams, the size of the eggs (80-100 μm) and the likelihood of mass spawnings, especially in the subtropics, would make cryopreservation a convenient means of utilizing gametes and safely transferring stocks around the region. The cost of cryopreservation and the success rate of the process for giant clams is worth investigation.

Conditioning

Broodstock conditioning has been discussed by Braley (1990) for *T. gigas* in Australia; attempts were made to spawn clams out of season by raising the ambient water temperature and fertilizing the water. This was not successful but methods of enhancing the nutrient input and altering the temperature regimes may be effective, as it is with other bivalves.

Various groups have used gonad biopsies to assess the reproductive state of individuals but this has not yielded consistent results. The selection of broodstock for spawning at the CAC is still a best guess method and relies on previous spawning records and visual assessment of gonad size and color. Spawning induction can consist of the following: general stress, temperature shock, intragonadal injections of serotonin and exposure to light. More detailed work needs to be conducted on conditioning and creating a gonad fitness index. Gallager and Mann (1986) demonstrated a significant correlation between egg lipid content and survival to the straight hinge and pediveliger stages of *Mercenaria mercenaria* and *Crassostrea virginica*. Such evaluation procedures could ensure that induction procedures only be used when broodstock condition is optimum.

Maturity

Based on known growth rates *T. gigas* takes approximately five years to become male mature and seven years to become female mature, *T. derasa* takes five years to become female mature (Heslinga et al. 1988). Such a lengthy maturity period translates into a slow selection process.

Triploidy

The advantages in producing triploid clams are unclear as yet. Although giant clams are very fecund they are slow in maturing and the optimum market size or age has not been fully determined. Triploidy may enhance growth rates, but the age at which energy is first diverted towards gonad development has not been determined. It is worth noting, however, that if triploid clams are similar to triploid pearl oysters which can produce viable gametes leading to aneuploid individuals (Wada and Komaru 1991), then the consequences on the natural population could be severe.

Zooxanthellae

The symbiont of the giant clam *Symbiodinium microadriaticum* is extremely

important in mariculture terms. The selection of various strains of zooxanthellae may have as much or greater impact and certainly a faster response time than the selective breeding of giant clams themselves. Strains of zooxanthellae affect growth performance in early life (Molea 1992). We keep a stock of our fastest growing clams specifically for sacrificing to extract the zooxanthellae to inoculate the larvae. Export of clams has to date included the export of zooxanthellae. It is possible, however, to export juveniles without zooxanthellae. Applied zooxanthellae genetics is an area that merits urgent attention.

Grading

Giant clams do not show a substantial growth difference in the first three weeks unlike other bivalves. By the fourth week grading is possible but initial trials indicate that size selective mortality of the smaller grades occurs rather than growth differences. A second grading is possible at tank harvest when the clams are 3-6 months old, different grades of this age group show different rates of growth and survival (Gervis, unpubl. data). Selection is therefore already taking place by virtue of the hatchery procedures. It is not known how hatchery-reared clams will fare in restocking programs.

Acknowledgement

The funding support provided by the British Overseas Development Administration is gratefully acknowledged.

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Discussion

MACARANAS: If you want to cryopreserve sperm how easy is it to collect them to do that?

P. MUNRO: They will settle out very quickly in a container if you collect them in sea water from a tank. You can of course also centrifuge them after collection, but then you must be careful of the flagella.

NEWKIRK: Cryopreservation of sperm has been done with *Crassostrea*.

The problems you have been talking about, viability, spawning, gametes, etc. all sound to me like problems associated with an industry or technology in a very early stage of development. These things will have to be dealt with if giant clam production is going to be viable in future. With oyster species, we had similar problems. We can talk about conditioning and some of these things but we have to hope these management problems are resolved.

HESLINGA: I agree with Dr. Newkirk in that some of the problems Mark is describing are not genetic. They're management problems, and they apply to *T. gigas* in a few situations. If we look at the other species, the smaller species are quite common and we simply don't have these constraints.

BRALEY: The conditioning is worth pursuing. One should use a medium for feeding the zooxanthellae, and take things out of the medium progressively to see what is necessary for the clams. We started to do this; we used basic fertilizer and Vitamin B1, but it obviously wasn't enough. You may need to consider also the water temperature.

Means to Identify Stocks and Strains

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MACARANAS, J.M. 1993. Means to identify stocks and strains, p. 25-29. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction

The definition of 'stock' as a management unit is somewhat arbitrary. In this discussion paper the 'genotypic stock' concept of Larkin (1972) is used, which defines a stock as a population having a degree of genetic uniqueness: "a population of organisms which, sharing a common environment and participating in a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed". This genetic stock, also called a local population (Sinclair 1988) is an evolutionary one because of the population's adaptation to local conditions. A 'strain' may best be defined from the present status of giant clam management as either a local, introduced, or synthetic population from either local or introduced broodstock. It may or may not be a genetically homogeneous unit, and the genotypic constitution of the resulting cohorts/batches/lines may vary with space and time as dictated by the limitations in broodstock numbers used in each spawning.

As a source of broodstock for either re-establishment of stocks or farming, giant clam stocks should be reasonably delineated over as large a part of their geographic range as possible, which is the Indo-Pacific region. The plasticity of shell and mantle characters (e.g., color, shape, size, etc.) discourage their use as markers in stock or strain identification. Distribution and differential life history patterns are other stock criteria that can provide important information for resource management. However, the criterion that can address the definition of a stock unequivocally and which should be used in conjunction with other sets of information, is genetic distinctness.

Genetic or Molecular Markers

Molecular variation, revealed by DNA and proteins, is stable because, unlike morphological variation, expression is not confounded by environmental effects. A review of 62 available articles on mollusc genetics from 1970 to 1992 shows that isozyme electrophoresis has been the major molecular technique used in investigating stock differences and genetic changes. However, recent advances provide alternatives for uncovering a greatly expanded number of genetic markers based on polymorphism of DNA sequences. Both approaches as applied to strain and stock identification are described below.

The Tools

Isozyme electrophoresis is the separation of protein variants by their differential migration under the influence of an electric current. These protein variants are called isozymes because they are alternative forms of the same enzyme and reflect mutations in the genome (see Fig. 1). This distribution of protein variants is the result of evolutionary processes (mutation, migration, drift, natural selection) accompanying the adaptation of the population to the local conditions. Its measure can be used to estimate genetic differences between populations. A large number of loci and individual animals can be examined in a relatively short time and at moderate expense. However, since protein expression is two steps away from the DNA code, it does not reveal all of the genetic variation that exists in the species genome. To illustrate, 64 codon combinations are available for coding 20 amino acids, so that a change in a DNA base will not

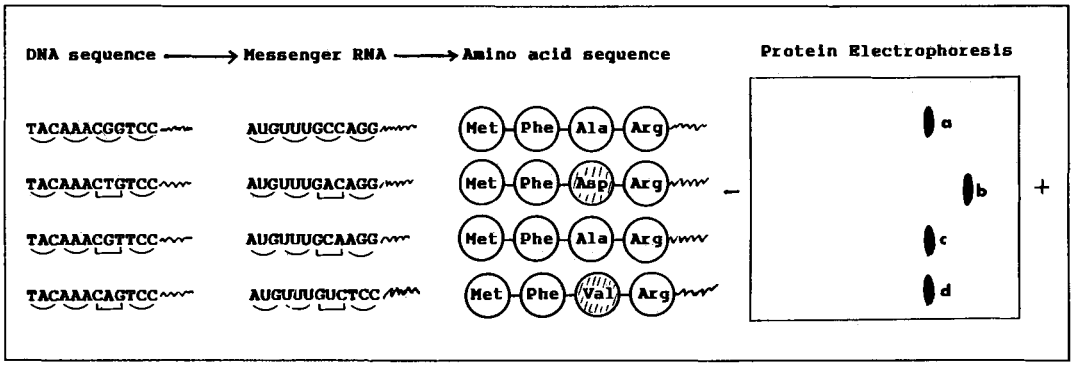

Fig. 1. The protein is the end product after DNA transcription and translation. A mutation in the DNA molecule can result in an altered protein (b) which exhibits a different electrophoretic mobility from the original protein (a); both proteins a and b are called isozymes. A change in the DNA code may not alter the amino acid sequence (c); even a change into an amino acid with a similar property may not change the protein mobility (d).

always lead to a change in protein structure. DNA base changes leading to amino acid substitutions may not alter protein mobility in an electric field. Moreover, staining techniques for isozymes limit the number of loci that can be visualized on gels.

Investigation of **DNA-level polymorphisms** for stock and strain identification can augment information already obtained from protein variants or can be applied to marker-based studies not answerable by protein variability (Hallerman and Beckmann 1988). DNA-level markers can be obtained from either the **mitochondrial** or **nuclear** genome. Mitochondrial phenotypes are maternally inherited (Hutchison et al. 1974) while nuclear genomic DNA exhibits Mendelian inheritance with co-dominant expression of alleles.

Both types of DNA can be cut at specific sites by special enzymes called restriction endonucleases to give "restriction fragments". Deletions or additions in a mutated gene lead to loss in recognition site for the endonucleases, resulting in different fragment lengths. This results in a type of polymorphism called restriction fragment length polymorphism (RFLP). Fragment lengths are estimated by comparing their electrophoretic mobility (which is largely a function of length rather than charge, unlike isozyme electrophoresis) with known size standards. In mitochondrial DNA analysis, each unique fragment pattern for a given restriction digest is identified and designated with a letter (see Fig. 2, X and Y). The most common outcome for intraspecific variation is to differ by three bands.

To maximize the information obtained from mitochondrial DNA genotypes, several

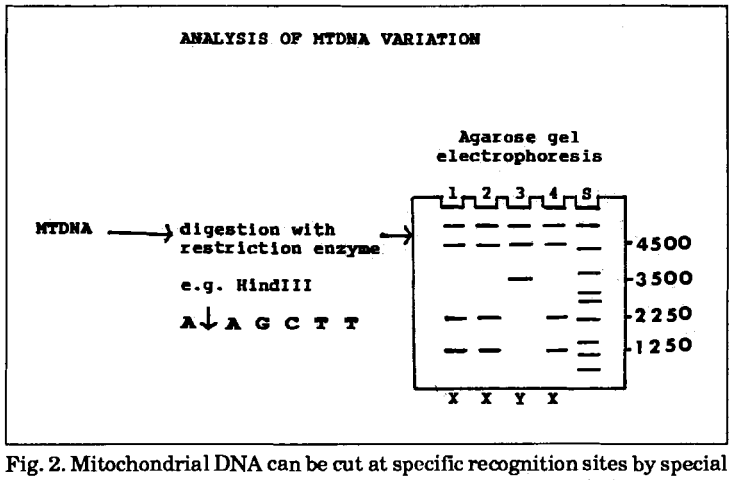

Fig. 2. Mitochondrial DNA can be cut at specific recognition sites by special enzymes called restriction endonucleases (i.e., the restriction enzyme HindIII recognizes the DNA sequence AAGCTT). In the example given sample 3 has lost one recognition site, and therefore has a longer fragment (3500) compared to samples 1, 2 and 4 which have two short fragments 2250 and 1250. Fragment size is compared with a standard (S) included in every run, and is expressed as the number of bases in the fragment, shown on the right. The two fragment patterns are arbitrarily designated as X and Y.

restriction enzymes could be used to cut the mitochondrial DNA molecule in question, possibly yielding population-specific fragments. Such a tendency for homogeneity in a population's mitochondrial DNA has been observed in pocket gophers, mice, and bluegill sunfish (review by Ferris and Berg 1987). The greater resolving power of mitochondrial DNA restriction fragment analysis compared to protein electrophoresis is a function of its direct genotypic interpretation (being the DNA itself), and of its higher evolutionary rate which is 5 to 10 times than that of nuclear DNA (Brown et al. 1979). Thus, mitochondrial DNA markers may not only be used in investigating stock structure; they are also useful in branding stocks or hatchery strains. It is therefore possible to monitor the success of a translocation or even hybridization by examining the genotypes of the recruits. The drawbacks of DNA-level markers techniques as compared to those of protein-level markers are that they are relatively expensive and are technically more demanding.

Nuclear or genomic DNA, in contrast to mitochondrial DNA, is constructed of many more base pairs and exhibits high levels of variation that cannot be matched by mitochondrial DNA or isozymes. A major source of genomic DNA variation arises from its complex structure of flanking, exon (coding) and intron (noncoding) regions. Most of the sequences in the introns and flanking regions are not represented in the final protein product and while some of these sequences are important in gene transcription, gene regulation and messenger RNA splicing, the vast majority are under no known selective pressure and are highly polymorphic (Whitmore et al. 1990).

The steps for analyzing genomic DNA are similar to those for mitochondrial DNA. However, genomic DNA is so large that any restriction enzyme digestion produces a multitude of fragments of various lengths, masking the electrophoretic resolution of single loci. Therefore a technique called Southern blotting is used, which allows the selective hybridization of radioactive DNA probes, consisting of complementary sequences, to appropriate fragments on the blot. A clear resolution of variation in fragment lengths (RFLPs) is seen on the autoradiograms (see Fig. 3). An individual who does not have the same restriction sites surrounding the gene sequence on homologous chromosomes is heterozygous; RFLPs appear to be co-dominant and dispersed throughout the genome.

Recent discoveries have described several regions of the human genome which contain a variable number of tandemly repeated oligonucleotides called VNTRs. The repeat units of some of the human minisatellites have a common core sequence which has been utilized by Jeffrys et al. (1985) to construct hybridization probes to identify hypervariable minisatellites after restriction enzyme digestion of the genomic DNA. The length of the restriction fragments is a function of the number of repeats in the allele. Because of the extremely high probability that two individuals will have

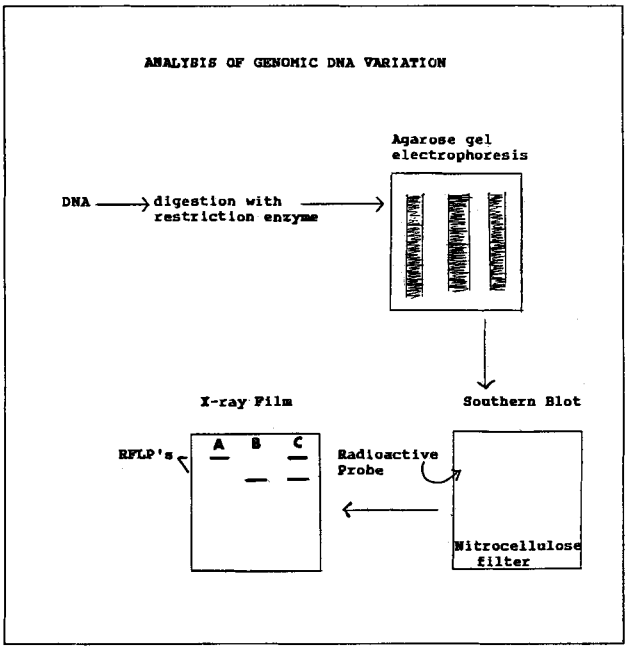

Fig. 3. Genomic DNA is digested by restriction enzymes in a similar manner to that of mtDNA; however, the large number of overlapping restriction fragments will result in smears. Restriction fragment length polymorphisms can be resolved by the use of probes with a Southern blotting procedure. The autoradiogram shows individuals A and B homozygous for two different restriction sites surrounding the gene sequence probed, while C is heterozygous for the restriction sites.

different numbers of repeats in each allele, the appropriate name of 'DNA fingerprint' has been given to the fragment patterns. DNA fingerprinting has been rapidly applied in forensic science, paternity testing, pedigree analysis, and to study breeding behavior in birds. Its potential use for marker-based technologies in fisheries science is apparent.

Sampling Strategy

Generally, the number and geographic pattern of localities that need to be sampled will depend to a large extent on the actual scale of substructuring within the species (Baverstock and Moritz 1990). For the Great Barrier Reef studies (Macaranas et al. 1992), some regions allowed two sites per reef (30 individuals per site) to be sampled, other regions had a scarcity of giant clams; single but reasonably sized (30-40) samples from the latter have been used for isozyme electrophoresis. Using mitochondrial DNA technology, the characterization of genotypes collected from a fewer number of individuals over the whole range of each species could provide a finer scale of subpopulation structuring.

Genetic Markers in Giant Clams

A biopsy technique for sampling mantle tissue was devised to prevent sacrifice of the clams (Benzie and Williams 1992; Macaranas et al. 1992). SCUBA divers cut a small piece of tissue from the mantle margin with surgical forceps and scissors while the shell was kept open with a wedge. From this tissue, as many as eight enzyme systems with significantly high levels of polymorphism could be investigated, namely: glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), diaphorase (DIAPH), peptidase using leucyl-glycylglycine (LGG), enolase (ENOL) and glutathione reductase (GSR). A detailed description of the sample preparation, electrophoretic conditions and staining recipes is provided in Benzie et al. (1993). While the use of mantle tissue alone has limited the number of genetic markers used in contrast to that of Ayala et al. (1973) and Campbell et al. (1975), comparable estimates of genetic variability were obtained.

Potential Applications of Genetic Markers in Giant Clam Management

The results of stock differentiation studies on several giant clam species using isozyme gene markers showed significant differences at the regional level. Using DNA technology, the characterization of mitochondrial DNA phenotypes on a few individuals collected over the whole range of each species could provide a finer scale of subpopulation structuring and consequently the identification of realistic management zones.

For restocking reefs, it may be important to use broodstock from the local region to maintain the natural genetic resources. However, alternative strategies have been used in regions where giant clam resources have been severely depleted. Translocations and/or mixing of gene pools characterize several strains presently being maintained, although their impact on recruitment has yet to be seen. The success of these practices can be monitored by DNA-level markers. Because of the maternal inheritance of mitochondrial DNA, it may be relatively easy to find unique markers for individual stocks. The observation of these markers in the recruits would establish which of the stocks had spawned or if hybrids had been produced. Thus, the effectiveness of stocking programs could be monitored. With regard to stock improvement programs, a particular marker would be extremely useful if it was linked to a desirable trait allele in the selected stock. Marker-based approaches might also be utilized in the investigation of disease-related traits.

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Discussion

BENZIE: A comment - one of the main logistic problems has been getting liquid nitrogen around the Pacific. With an improvement in technology, there is the possibility that you could get alcohol-preserved samples for the DNA work. I wouldn't want to place a whole sampling program on that assumption at the moment, because the results are still a bit inconsistent using alcohol to preserve the material. As regards getting more information using DNA, especially using nuclear DNA, the comments that you can make are restricted by the same statistical analyses as proteins are. With nuclear DNA you can make all sorts of comments on gene flow etc., but you've got to be very careful about the kinds of questions that you're asking.

EKNATH: We have reached the stage in our tilapia genetics program where we want to be able to put a DNA marker in the fish that we are disseminating. An optimistic view is that we'll succeed in identifying our fish 20 years from now using a genetic marker.

BENZIE: It's a fundamental requirement for any genetics program to maintain a good database. An example of how difficult it is - if you look at a well-established industry like the Australian cattle industry - they recently did some DNA fingerprinting on one of the breeds and they found that their records did not match the fingerprinting results. Of course this is after artificial insemination was introduced. It's a very crucial issue, and even in a sophisticated industry like cattle, the information records can be defective.

General Discussion 2

The discussion centered on two perceived goals: to maintain biodiversity and to breed a 'superclam'. The geneticists pointed out that maintaining biodiversity could not be a goal in itself, because the necessity to maintain genetic diversity applies both to farming and to re-establishing stocks; the variation is needed to be able to select artificially in a farming situation, and also for natural selection to take place without deleterious inbreeding effects.

It was agreed that a desirable situation would be to have protected areas for wild broodstock in each of the regions identified by John Benzie as having genetically distinct populations. Gerry Heslinga stated that it would not add significantly to the costs of an established hatchery to maintain wild broodstock, as the animals are self-feeding; and that most Pacific Island governments lack the financial resources to set up and maintain reserves such as the GBR in Australia. Roger Pullin pointed out that as in all other kinds of farming, maintaining biodiversity could not be left to the private farmer, whose priorities are economic. John Munro suggested that Pacific Island governments might consider combining refuges for giant clams with marine parks for tourism and diving, as Solomon Islands government has done on a small scale at ICLARM's CAC and Nusa Tupe field station. He also pointed out that hatcheries and farms are separate, and that all good hatcheries would try to maintain a

large pool of broodstock. *(The problem of disease spreading in a large aggregation of clams within a small area was not discussed, but has since proven to be pertinent. Ed.)*

John Benzie stated that farming and re-establishment of stocks with their natural genetic diversity conserved are two quite divergent goals, and that the second goal would be very difficult to achieve.

The problem of re-establishing stocks in areas such as the Philippines with very low numbers was discussed; the consensus was that it would be best to collect clams from a wide variety of places within the region of gene flow, in small shipments of nonselected batches with different parents, and at the same time to try to maintain the relic stocks so that their genotypes could be integrated with those of the exotic stocks at some stage. It was stressed that each situation will be different, and that where there is an undisturbed pristine population, this constitutes a valuable resource, and that one mélange of everything is not the desired objective.

Dr. Eknath said that there was no problem in breeding better clams for the farmer to grow, given the genetic variation in the base population to begin with. Furthermore although giant clams are seen as having a long generation time, it is worth remembering that the salmon breeders started only 18 years ago, and now about 70% of their breeders are fast-growing. Salmon have a generation time of 4-5 years, which is comparable to that of giant clams.

Consideration was given to the question of importing a variety of zooxanthellae in order to have diverse stocks of symbionts. However it was pointed out that the genetics of zooxanthellae is far from clear at this time, that different strains/species exist, that hosts select these different strains in a manner which is not understood, and that evidence from the CAC suggests that it would be preferable to ship clams without zooxanthellae as they continue to be able to take up symbionts at least up to 38 days, and thus a potential source of disease infection is eliminated.

John Benzie mentioned the option of gene banks, which although expensive to maintain, would be a complementary approach together with reserves in preventing the extinction of giant clams through overfishing. However he stressed that where only a hundred or so individuals are left, over 50 or 60 years it would be difficult to maintain enough variety in the gene pool for the survival of the species.

Plenary Session
Guidelines and Recommendations

A. Guidelines

Two sets of guidelines were drawn up by working groups and discussed at the final plenary session of the workshop; one set dealing with practices based on sound genetic principles for hatchery managers, and the other dealing with the genetic implications of translocations.

1. Guidelines for Hatchery Managers on Sound Genetic Practices for Cultivation of Giant Clams

a. Present hatchery procedures eg grading (up to about 6 months of age) within batches, use of antibiotics, fertilizers, feeds etc., probably do not affect genetic variance. As in other bivalve species the quality of the eggs is probably a paramount factor in initial growth performance. Care should be taken however not to reduce the variance of the family size when broodstock replenishment is done, therefore as many parents as possible should be used to produce the F1 generation. It was recognized that it is not efficient to maintain runts in the limits of hatchery space, but that research into the impact of culling and other hatchery practices needs to be done (see section B below).
b. Clear records of spawning regarding parentage should be kept, and these records should be standardized. Traits of economic importance, e.g., growth rates, should be recorded, and a database should be developed and maintained.
c. Some individuals from each successful spawning should be maintained. Representatives from as many batches as possible, each from as many parents as possible, should be maintained in each hatchery.
d. The terminology used in giant clam cultivation, e.g., batch, cohort, family etc., should be standardized, and advice from the ICES Working Groups on Genetics Mariculture Committee will be sought. (In the meantime, "line" should be avoided).

2. Guidelines to be Adopted for Translocations

For all that follows it is assumed all translocations are subject to standard environmental and quarantine procedures.

a. It is strongly recommended that a code of practice be developed to standardize environmental and aquaculture procedures.
b. Transfers for re-establishment should be accompanied by detailed records of source, constitution, parentage (including identity numbers) disposition, and destination; and these records should be maintained in a central database.
c. Introduction of exotic species should be effected only when all necessary precautions have been undertaken and in accordance with accepted international protocols. A thorough assessment of local stocks should be made before introductions are considered.
d. Where translocations are effected to depleted areas, all relic stocks should be tagged and identified and, where possible, reproduced to maintain their genetic identity.
e. International introductions of conspecifics to areas with abundant wild stocks should be discouraged.
f. For the purpose of re-establishing a stock, the largest genetic diversity should be sought. Successive cohorts imported from a given source should be small and derived from different parents on each occasion.

B. Research Needs

The following research needs were identified by a working group and then discussed at the plenary session.

1. Genetic markers to be developed for identification of stocks, firstly to keep track of stocks, and ultimately to try to correlate markers with quantitative and qualitative traits.
2. Evaluation of the strains of zooxanthellae in tridacnids, the number of strains involved, their differences and distributions to be investigated.
3. Definition of desirable traits (e.g., economic), their genetic variation and heritability to be studied.
4. Characterization of the natural stocks, phenotypically and genotypically (Benzie's group, see p. 1, has already started on the genotypic characterization of stocks in the Pacific). Thorough assessment of local stocks should be done before pressure arises to make introductions.
5. Genotype-environment interactions to be investigated: firstly considering the zooxanthellae as part of the environment, and then other environmental factors such as location, offshore/inshore etc. to be considered as factors affecting the performance of cultivated giant clams.
6. Assessment of the genetic impact of hatchery procedures, e.g., culling, grading at various stages to be made.

(Research topics not discussed at this session but identified during the workshop were: investigations into broodstock conditioning, and the possibility of applying cryopreservation techniques).

C. Support Facilities

Analytical services for the development and use of genetic markers could be provided by the Australian Institute of Marine Science (Dr. John Benzie), and by the University of the Philippines Marine Science Institute (Dr. Edgardo Gomez) in the short term. In the long term, other participating institutions may be able to analyze their own stocks using DNA methodology.

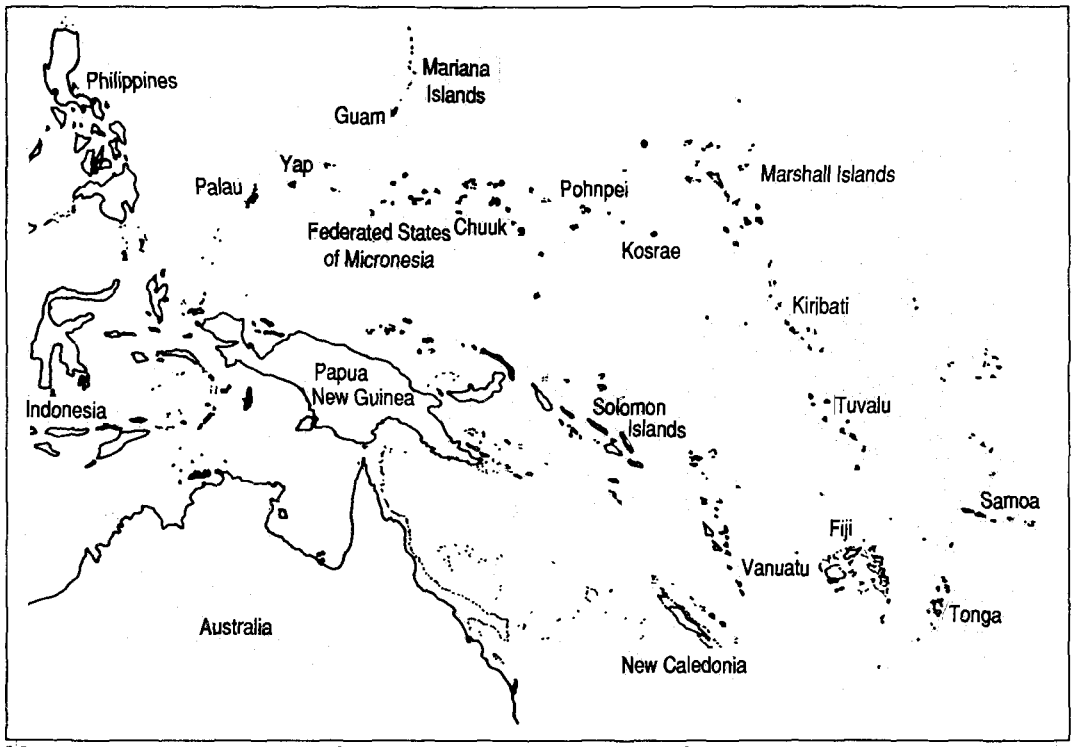
A central database of international translocations should be developed and maintained by ICLARM. All participating institutions to be enjoined to contribute to it and to maintain their own internal and **compatible** databases.

Clamlines (produced by the CAC) should be expanded to serve as a vehicle for the communication of news and information on giant clam genetics.

D. Giant Clam Genetics Consortium

It was decided to seek funding for the re-establishment of giant clams in the Pacific Ocean in a manner which conforms to sound genetic principles. To this end a consortium was formed, consisting of representatives of the various institutions attending the workshop. A proposal for funding the re-establishment is to be submitted to various agencies, and it was agreed that there would be no objection to ICLARM administering any funds obtained for this purpose.

COUNTRY REPORTS



Map of the West-Central Pacific showing place names mentioned in the country reports.

Federated States of Micronesia

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LINDSAY, S. 1993. Federated States of Micronesia (country report), p. 33-34. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

All of the islands within the US Affiliated Pacific group have received giant clams from the MMDC at some stage over the past 12 years. Restocking is a major goal of these islands. As well as *T. derasa*, *H. hippopus* and *T. gigas* are used, but to a lesser extent. Local staff, generally from the various Fisheries Divisions, have undergone training courses at the MMDC.

Kosrae has a hatchery, which has been set up to restock the reefs of the four States of the FSM. Kosrae has only *T. maxima* left at this time. We have 18 *H. hippopus* broodstock. We lost 25 in the last 4 months to rickettsia. We had 6-7 year old *T. gigas* brought in from the Marshalls - not old enough to breed. We have had a couple of spawnings of *T. maxima*; the settlement was very poor, for some reason every batch has died at the 2-day veliger stage. Kosrae is a high island, as is the main island of Pohnpei and there are only very small areas to grow clams. We pump water from the "blue hole" which is a region within the reef. Road building in Kosrae now may account for the poor water quality. The coral has died back to 60% of what it was this time last year. We're also producing small numbers of trochus and the local greensnail. These are used as training material to teach larval techniques. We shall bring in more broodstock from the Marshalls, possibly from Pohnpei.

Kosrae sent the first shipments of *T. derasa* to the other states of the FSM in 1992. The FSM states Chuuk, Yap, Kosrae and Pohnpei have all received 3,000 *T. derasa* (1.4 year olds) from Kosrae hatchery in 1992. The clams are to be used as each state sees fit,

e.g., Pohnpei is marketing theirs, while others are using theirs for re-seeding. Several training courses are being planned.

Pohnpei has a small hatchery which was designed to produce trochus. They have brought in mainly *H. hippopus*, and still have some of this species naturally occurring on the remote atolls. They are setting up 3-5 small growout farms, which are selling their clams to two local Japanese restaurants. Assistance and clams are provided by the Marine Resources Division. Eventually clams will have to be purchased from the Marine Resources Division, and less help will be available to the farms. Theft from the hatchery has been a problem in Pohnpei.

Guam has an aquaculture facility, and *T. derasa* supplied from MMDC. The numbers are currently low due to cyclone damage in early 1992. They have no breeding program yet, and the clams are kept in a land-based facility.

The Northern Marianas has no aquaculture hatcheries, and small numbers of clams, less than 500, supplied by the MMDC. The environment is not a good one for giant clams.

Chuuk has *T. gigas* and *H. hippopus* for re-seeding, as well as the *T. derasa* received from Kosrae and MMDC. They have stocks of *T. maxima*, and some *H. hippopus* and *T. squamosa* left on the outer atolls.

Yap has the largest remaining stocks of introduced clams, mainly *T. derasa*, but also some *H. hippopus*, all from the MMDC. Yap has large numbers of adult *T. derasa* on their reefs now, and they also have some natural stocks of *H. hippopus*. They are planning a 2-3 week survey to see whether recruitment is occurring. There is one report of a small *T. derasa* on Yap proper. A short survey (12 hours in the water) found no evidence of recruitment in 1991.

Marshall Islands has three hatcheries, two private and a government one. The private hatchery that produces reasonable numbers is Robert Reimers' Enterprises; they have 30,000-40,000 one year old *T. gigas*, 40,000-60,000 *T. maxima* 3-7 months old, and 5,000-10,000 three month-old *H. hippopus*, on an atoll called Mili. The Marshalls have several atolls with very good stocks, and Mili is the best. The other small hatchery on Mili was producing 500-1,000 clams a year, but has closed either temporarily or permanently. They are unique in that everything they do is in floating cages. Mili has *T. maxima, H. hippopus,* and *T. squamosa* in large numbers still. Robert Reimers' Enterprises is producing *T. maxima* for the aquarium trade, *T. gigas* (approximately two years old) for Japanese restaurants, and *H. hippopus* for re-seeding programs. The third hatchery is the government one on Likiep atoll, now it is a skeleton hatchery only, but it should be operational by the end of 1992. A training course will be given there in January 1993.

American Samoa has a hatchery, again it has very few natural clams on the reef, some *T. maxima*. Their broodstock is from Palau. They have just over 400 eight year old *T. derasa* which they have spawned. There is a remote atoll associated with American Samoa, where they would like to get some more *T. maxima*. However the US Fish and Wildlife Service will not allow this as it is classed as a reserve. They have a restocking program, and have several thousand juvenile *T. derasa* in nursery sites.

On Kosrae we have a problem with people stealing clams. Broodstock left on the reef there may be taken, and clams are still taken from our tanks at night. That is also the case for American Samoa. They brought in broodstock for spawning and people jumped the fence and stole the 30 broodstock. Now they have security guards.

The people on these islands have no idea of genetics whatsoever and they will take clams from wherever they can. Quarantine procedures are basically non-existent. In each place where clams are received they generally go straight out on the reef, not into quarantine tanks. This is not so in Kosrae and at MMDC. Quarantine procedures will be implemented in future in all places.

None of the government hatcheries is commercial, they are concerned with restocking only. The farms are generally looked after by individual people co-ordinated by the Marine Resources Division in each of the States. Most people have an arrangement whereby in five years' time they will get 50% of the clams and the rest will go back to Marine Resources. In Kosrae we will claim back 60% for broodstock.

Discussion

HESLINGA: The habitat these animals occupy is prone to cyclones, so that any facility can expect to be damaged by a cyclone within a decade or so. We lost 100,000 clams in a cyclone about 2 years ago - 155 mph winds, broodstock were even moved about on the bottom. We suggest a gene bank to build in a safety factor.

BENZIE: Gene banks are a sensible strategy in any case with respect to disease. In the salmon breeding programs in Norway for example a lot of effort is going into setting up second breeding stations as a fall-back position in case of diseases.

HESLINGA: The MMDC is applying for "Captive Bred Status" from CITES. This is essentially their recognition that we have closed the life cycle and are no longer heavily dependent on the natural environment. One of the requirements is that there must be a second institution in the US which produces the same animals to the same level of proficiency. This has delayed us, but now American Samoa is beginning to produce *T. gigas* consistently, so that US Fish and Wildlife is now willing to accord us that status.

Australia

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BRALEY, R.D. 1993. Australia (country report), p. 35-36. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Status of Stocks. Six of the eight species of giant clam are found in Australian waters. These include *Tridacna gigas*, *T. derasa*, *T. squamosa*, *T. maxima*, *T. crocea*, and *H. hippopus*. Stocks of all of these species are in good shape, due to the extensive coral reef habitat, especially the Great Barrier Reef (GBR) of NE Australia, which has the largest stocks of giant clam in the world. Only a small number of Aboriginal people eat giant clams as a staple or special food. Giant clams are now protected by law from collection for food, except for traditional food of Aboriginal peoples.

In the 1960s and 1970s, foreign fishing vessels poached considerable numbers of clams from GBR reefs. There were an estimated 69,000 *T. gigas* adults poached from north of Cairns in the early 1970s. Despite decimation of natural stocks, there are good numbers of stocks on many reefs. There are latitudinal limitations on natural stocks. This is most apparent with *T. gigas* which has natural breeding populations limited to north of about 18°S. The limiting factor appears to be low winter temperatures which stress and kill young juvenile clams of this species. At Orpheus Island Research Station (OIRS), a lot of juveniles were therefore selected on the basis of surviving the cold temperature.

A large operation to relocate thousands of 6.5 year old cultured *T. gigas* to various reefs for long-term experiments, for tourist operations, and for aquaculture broodstock use took place in late May 1992. About 5,500 clams were moved with 90-92% survival. This involved the Australian Navy, the Great Barrier Reef Marine Park Authority and James Cook University (with AQUASEARCH consulting for JCU). Some clams were out of the water for 30 hours in tanks on the decks of the Navy landing ship. Fire hoses spraying on deck were used to keep the clams wet. Part of this exercise was aimed at simulating a large-scale recruitment at a chosen 'spawning/larval source reef' to look for computer-modelled connectivity to 'larval sink reefs'.

A 5-year resurvey of the largest natural recruitment of *T. gigas* on the GBR was completed at Lizard Island and nearby islands/reefs in late April/early May 1992. Survival from 10-cm shell length juveniles in April 1987 to this resurvey was 56% at one site and 9% at the other site (mean shell length was 41.8 cm). Most of the clams were found along the edge of the channel where the branching *Acropora spp.* is located. At Lizard Island there are two smaller islands which encompass the lagoon. Measured from the center of the lagoon to the outer reef there is SE direction Island, NE direction Island and Iso Island, each five nautical miles from the center of the lagoon. We went out to each of these reefs and measured all the recruits we could find. They were within the size class of the spawning at Lizard Island itself. This gives some indication that the spawning took place in Lizard lagoon.

Facilities for the Culturing of Giant Clams. OIRS has ceased as a giant clam culture facility. However, it could be used again to culture giant clams given the financial support. Reefarm Pty Ltd, based on Fitzroy Island, continues to produce giant clam seed, mainly *T. crocea*, as well as other marine organisms. Reefarm obtained a permit in 1991 for an ocean nursery/growout site at Arlington Reef of 10 ha, given that the pilot exclosure system is successful. The main species being reared thus far is *T. crocea* for the aquarium trade and for pilot trial shipments to Taiwan or Japan. Pacific Clam Pty Ltd was based at Sudbury Reef, not distant from Fitzroy Island. Cyclone Joy (December 1990) destroyed all of the ocean nursery facilities, but poor survival of juvenile clams even prior to this time may have led to the demise of this company. There is a father/son giant clam farm being set up in Western Australia. AQUASEARCH is holding cultured F1 *T. gigas* and *H. hippopus* at two oyster leases (Great Palm Island and Magnetic Island) for future broodstock usage. Unfortunately, all are from one spawning at a private company, but AQUASEARCH hopes to get others from batches spawned at OIRS.

If giant clam is reared in an Australian national park property, which Orpheus Island is, it cannot be used for any commercial gain, even as broodstock. There is a research site being kept in Hazard Bay, Orpheus Island for long-term monitoring.

Future Plans. This will depend on the success of the private mariculture operations. AQUASEARCH plans to spawn F1 clams in about five years. James Cook University will maintain an interest in giant clams and there may be graduate or honors students who will continue to use some of the clams reared at OIRS.

Discussion

HESLINGA: What might be the genetic consequences of releasing those cultivated clams on the GBR?

BENZIE: It could be quite immense. The populations that we looked at on the GBR showed no genetic differentiation. You could conclude that the gene flow is so great that you don't need to worry about a perturbation at any one site, as it would be sorted out by gene flow from other sites. But if you have a large perturbation particularly upstream (there's a southerly current at the time the clams breed in the GBR), and you therefore have a large pulse of genetically different clams, and it coincided with a year of major recruitment, you could have a very rapid change in genetic constitution throughout the GBR.

There is a good model for this: the crown-of-thorns starfish. They produce a large number of larvae, and the larval lifespan is a little bit longer than that of the giant clam. It takes a very small shift in either the survival or the production of larvae to go from very few animals to a huge plague. So there is the potential for a considerable flow of material through the GBR.

BRALEY: I might just explain that these animals were put very close to the southern end of the GBR. Natural populations of giant clams are not found south of this area. As I explained they had already been selected for survival in colder weather at Orpheus, so they may survive the winter well.

HESLINGA: What's the worst that can happen? Let's assume that the clams released have a slightly lower heterozygosity than wild ones. Will they be less well-adapted? Would their survival be lower?

BENZIE: No, it's more complex than that. Selection occurs all the time whatever populations are involved. Stochastic effects are involved here. I don't think you can say that because a load of animals survives they are as good as or better than the natural population, because you're talking about a time scale of only a few years. The natural population has been there a lot longer. The potential for genetic change is there and may have a long-term effect.

HESLINGA: Let's assume that the released animals are breeding prolifically and influencing the existing gene pool. I'm not certain that's a bad thing.

BENZIE: We must look at what has happened with wheat and its relatives in agriculture. We have wiped out a lot of variation by plant breeding.

HESLINGA: On the other hand look at chickens. All over the world we find domesticated chickens, not wild chickens. Is that a good thing or a bad thing?

BENZIE: It depends very much on the time scale that you're looking at. If you're looking at 100 years say since the Industrial Revolution, it may not matter very much. On a longer time scale it may be absolutely vital that you maintain genetic variation.

HESLINGA: People will always have an effect on the wild populations.

Solomon Islands*

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OENGPEPA, C. 1993. Solomon Islands (country report), p. 36-38. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Introduction. Six tridacnid species, namely *Tridacna gigas, T. derasa, T.maxima, T. squamosa, T. crocea* and *Hippopus hippopus* are found in the Solomon Islands. Field trips made between 1987 and 1991 by ICLARM staff confirmed that stocks of *T. gigas* and *T. derasa* have been severely depleted in many areas, including Marau, Russells, Savo, Kia Nggela and Marovo (Govan 1987a, 1987b, 1988, 1989a, 1989b, 1989c; Govan et al. 1988). The trend is continuing at an alarming rate. The decline is attributed to overharvesting by coastal dwellers.

Giant Clam Production at the Coastal Aquaculture Centre. Broodstock at the Coastal Aquaculture Centre (CAC) have been collected from four different provinces; Central, Guadalcanal, Isabel and Western Provinces. To date, the number of broodstock is 76** *T. gigas* and 45 *H. hippopus*. In addition to these we have a small number of *T. derasa, T. maxima, T. squamosa,* and *T. crocea*. *T. gigas* that have spawned eggs have ranged from 38 cm to 90 cm shell length (SL). The largest number of eggs collected at any one spawning was 240 million from a 77-cm *T. gigas*.

The CAC has eight fiberglass larval rearing tanks (6 x 700 l and 2 x 1,500 l) with a total holding capacity of 216 million eggs, when stocked at 30 eggs/ml^{-1}. Larvae are reared in these static tanks with light aeration for 8 days after which they are transferred to

*ICLARM Contribution No. 922.

**At the time of writing. In late 1992, most of these were killed by disease of unknown origin.

four outdoor 2,500-l plastic-lined tanks, in which stocking densities are 5 veligers/ml^{-1}. Veligers are kept in these tanks for about 2 weeks to age 21-28 days and then stocked into settlement tanks. The tanks are constantly supplied with fresh seawater with a 100-mm pump.

Over the past five years, we have raised larvae from 17 spawnings of giant clams, 11 of *T. gigas* and 6 of *H. hippopus*. During 1991, a total of 503,000 spat were produced. These clams were transferred to floating ocean nurseries (FONs) at a stocking density of 5,000/m^2 for 10-mm clams and 15,000/m^2 for 3-mm clams. The CAC's FONs produced 134,000 35-45 mm clams in 1991 of which 113,000 were transferred to Nusa Tupe Field Station in Western Province and the remaining 21,000 to village trial sites.

Clams of 2.7-mm mean shell length (SL) are transferred from land-based nursery tanks to FONs but optimum size of transfer appears to be around 3.5 mm SL. This permits three cohorts to be raised in each nursery tank each year.

Growth rates of *T. gigas* in FONs at the CAC have averaged 5 mm·month^{-1} for clams over 10 mm. The survival rate of clams between stocking and harvest showed that clams stocked at 10 mm or more survived better, ranging between 30% and 70% while clams stocked at 3 mm ranged between 20% and 30% survival. Clams are harvested at mean length between 30 to 40 mm.

Growth rates at Nusa Tupe have averaged 2-4 mm·month^{-1} in cages, 3-5 mm·month^{-1} in FONs and 5-6 mm month^{-1} in exclosures. Growth rates of tagged individual clams in cages and exclosures have been 0-6 mm·month^{-1} and 0-10 mm·month^{-1}, respectively, the majority averaging 3-7 mm·month^{-1}. Survival rates of *T. gigas* have ranged between 10% and 50% for clams grown from 10-12 mm to 30-35 mm and from 20% to 60% for clams grown from 35 mm to 100 mm.

Community Participation. Village ocean nursery trials have been developed to investigate the viability of community participation in giant clam farming. Of the 22 trial sites that have been established, 17 are currently active. Survival in these sites varies, ranging between 0 and 85% for clams supplied at 30-50 mm. The best survival and growth rates (averaging 7 mm month^{-1}) in *T. gigas* have been achieved using highly selected clams placed on or close to reef slopes and adjacent to channels with a moderate to strong current. An average growth rate of 3-5 mm per month was observed on reef flats and areas with low current flow.

At three-month intervals, CAC staff responsible for the ocean nurseries visit village trials. During visits participants are given the opportunity to discuss innovative ideas or difficulties encountered. Villagers are advised to clean cages and remove predators (mostly ranellid gastropods) at least twice weekly. At a mean length of 100 mm, clams are transferred into exclosures, erected with the help of CAC staff on routine tour. Cleaning and removal of predators from exclosures are carried out on a weekly basis.

Harvests and Markets. Interest in exporting giant clam meat and shell from the Solomon Islands has increased, and greater pressure on *T. gigas* stock is anticipated from licensed commercial harvesting as well as poaching. According to the Fisheries Division, a total of three companies have recorded their interest in exporting clam adductor muscle.

Prior to 1991, giant clam products were classified as "other exports" and there is no indication of the quantity exported. According to the 1991 Fisheries Division report, a total of 1,133 kg of *T. gigas* adductor muscle (from approximately 2,000 individuals) was exported to Singapore. This was purchased locally by the dealer at US$3.63/kg and exported at US$7.26 (including FOB). The total export value was US$8,218.87. Additionally, the Statistics Division recorded that in April 1991 shipments of giant clam products were exported to Australia valued at US$14,157.94 (including FOB) and in November to Singapore valued at US$2,042.07 (including FOB). Confirmed export market figures seem low compared to reports received from local people. Poaching in isolated, remote islands and outlying reefs cannot be excluded. There has been confiscation of giant clam adductor muscle harvested by Taiwanese poachers at various times.

In Solomon Islands, *T. gigas, T. squamosa, T. crocea, T. maxima* and *H. hippopus* are sold in urban markets, mainly in Honiara. The whole meat is sold at a price ranging between SI$1.00 to SI$5.00/kg. The shells have long been utilized as inlays on carvings, bracelets and other artifacts.

Conclusion. Stocks of six tridacnid species found in Solomon Islands are being depleted at an alarming rate. To ease this pressure of overexploitation, there is good reason to encourage community participation in giant clam stock management.

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Discussion

HESLINGA: Is there a general assessment between floating ocean nurseries and bottom-based culture - what is your feeling on which is more viable?

J. MUNRO: The FONs are actually a substitute for the land-based stage because we get them out of the tanks at ~3.5 cm. That means we can get more through each tank per year. The comparison is therefore really between the tanks and the FONs. The costs are similar overall, but the growth rate is enhanced in the ocean. We do still use trestles.

Palau

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The goals of our program are: 1. to maintain natural stocks if possible; 2. to preserve biodiversity; 3. to domesticate giant clams.

The question in the Indo-Pacific is how realistic is it to try to maintain natural stocks - in some areas it may not be realistic at all. Our second goal is essential for the preservation of the genetic resources necessary for the maintenance of natural stocks and for farming. Our 3rd goal - giant clam is extremely marketable, and we want to turn it into a domesticated animal. Are these three goals always compatible? Maybe heavy investment in terms of manpower, time or funding is not realistic in all these goals.

In Palau we have all seven species of giant clam except *T. tevoroa*. Our impressions are that while the numbers are not as high as they were historically, they are relatively high compared with some islands in the Pacific. It is still possible to collect ~200 lb of *T. crocea* in a morning without getting into a boat. As for *T. gigas*, there used to be 10-15 per hectare in some areas, but now there aren't that many. However it is not extinct.

Marine reserves and sanctuaries are being set up by the government of Palau, in conjunction with international bodies like the Nature Conservancy. As elsewhere, the problem is to enforce the legislation. The clams are popular as food, and people eat them daily. So they are aware of the need to cultivate them and to conserve them.

The MMDC has been in Palau for 15 years and the giant clam project has been active for 10 years. We have good local support and serious production began in 1984.

The hatchery is based on a modular system of concrete tanks, used both for larval culture and for nursery culture up to an age of ~12 months. It is an intense cultivation system with rapid turnover of water, heavy aeration and ammonium nitrate added on a daily basis, and sometimes phosphate. Spawning tanks are inside the hatchery building, and all spawning is done indoors. Larval rearing is done indoors using a variety of methods ranging from selected larvae to extensive cultivation. Fertilized eggs are put directly into culture tanks. We use splasher pools for larval culture and also for juvenile culture in the land nursery. An innovation is the use of marine mesh for building splashers - it doesn't corrode so it is indestructible.

Our seed production is enhanced by aerating the raceways. We found that aeration can be used not only to put more oxygen into the water, but also as a tool to control recruitment density in the settling tanks. The juveniles used to go to the edges and the middle space was wasted. They will settle along the airline, and we use this to better utilize our tank space.

Our ocean nursery is adjacent to the MMDC. We have about 2,000 bottom cages. We have gone from using fiberglass trays with a plastic mesh to using a metal box of PVC-coated 14 gauge wire. This is resistant to the wrasses and big pufferfish and rays, etc. Fouling used to occlude the mesh (1 inch), we now use 2-inch mesh and put the clams out when they are about 5 cm. The meshes are therefore large enough that total occlusion never occurs.

As soon as we had a production capacity we created several lines of broodstock. We produced about 10,000 broodstock which we set aside until they were 8 years old. Of the 10,000, we culled the slower-growing 5,000 and sold them to Okinawa as meat. We have a large pool of *T. derasa*, at least four

distinct lines 10 years old and then we have many other lines that are younger from which to choose when we spawn. The lines are kept distinct. While we don't tag individual animals, we do keep cohorts separate and track them carefully throughout their entire lifespan, whether they are sold, exported or kept as broodstock.

We have closed the life cycle of *T. derasa*. We have produced F2 *T. derasa* beginning in 1984 using F1s that were produced in 1979. We now have several cohorts of F3 *T. derasa*. We have about 4,000 *H. hippopus* raised to maturity. They produce eggs at 3 years of age, *H. porcellanus* at about 4 years produce sperm and eggs, we have about 2,000 of those F1s and about 3,000 broodstock. All other species are being produced including *T. gigas*, but to a lesser extent. We had a large cohort of *T. gigas* through about 2 years ago. About 10,000 remain, and they're scheduled to go to Yap soon.

Our interests include training and technology transfer, which may involve donation or sale of clams. We have donated 2,000 lb of broodstock to each of the 16 states of Palau. We have also been active in attempting to establish reserve areas all over Palau, as well as many of the Pacific Islands.

Our primary objective is no longer research and development, we have been at that for 10 years. Now we have turned to marketing. For the interest of the industry it's important that we demonstrate that all this effort that we're putting into raising clams can be used to some benefit, either nutritional or to create business for profit. We're still a government facility, we still actively seek research grants, but we are self-sufficient, based on what we export, and what we sell locally. If the granting agencies were to dry up and blow away as they sometimes do, we would still be in business. We're talking about animals that have a generation time of 5-10 years, and if we want to be successful in what we do, we have to take a long-term approach.

We are involved in supplying seed clams to other Pacific Island governments for whatever purpose. Some are interested in stock enhancement and some are interested in small-scale farming projects. We are into commercial farming based on our local and export sales. We have been exporting sashimi to Okinawa during 1991 on a weekly basis, 100 kg per week at $15/kg FOB. We have been selling *T. derasa*, 7 years old, the entire meat minus the kidney. We sell the baby clams as aquarium pets in the US; *T. derasa* is now quite well known in the US aquarium trade. We're also supplying *T. gigas, H. hippopus, T. maxima* and *T. squamosa* to those markets.

Adductor muscle is obviously of interest to some programs, but not specifically to the MMDC, except as sashimi, for which we sell mantle and adductor, raw or chilled. But our primary aim is not to produce adductor for international markets. Our opinion is that if you do that many other marketing opportunities will be missed.

We're actively involved in marketing shells, both locally and internationally. We set up a little gift shop at the MMDC, and we sell unworked shells for $8 a piece (*T. derasa* 6-7 years old), and we make a variety of handicrafts - soap dishes, ashtrays, wasabe dishes at $5 are very popular with Japanese tourists and we supply them to the local restaurants too. We put a lot of effort into making sure the colors are good because that's what sells. We also produce jewelry from baby clams, two kinds of earrings and shell pins and key chains. We supply the local Duty Free shop, which is a worldwide chain.

Our annual income includes sales of clam seed, revenues from training programs, and income directly associated with giant clam products, and it went from 0 in 1984 to $100,000 in 1989. In 1991 we grossed $180,000 which exceeded our projections by $40,000. In 1992 we expect to gross more than $200,000. It costs us about $150,000 to run the project, so by any measure we're into the black. The project is viable and self-sustainable.

As for problems - they are basically bureaucratic. We're dealing with the US Fish and Wildlife Service over the CITES issue. Palau is a trust territory under US jurisdiction with respect to endangered species. Every international shipment we make has to be inspected by a Federal agent, who may be uncooperative, and who also has to be paid by us. Most of us here are facing the same problems of being in remote places, where shipping and airfreight costs are expensive. Sometimes there are corrupt freight forwarding agents and customs officials - it happens everywhere, not just in Micronesia. The effect is a constraint to trade.

Discussion

EKNATH: You say that you have closed the life cycle and that you're on the route to domestication of giant clams. Where are you going to now? Are you going to invest in genetics?

HESLINGA: We came here to learn more about genetics.

LINDSAY: Are you going to mention anything about your reseeding or are you going to leave that to me?

HESLINGA: We have sent clams around Micronesia, also the Philippines, and there are many transfers going on. There is the potential for great benefit as well as harmful effects. As farmers we are modifying the environment. Tilapia is the aquatic chicken, we see no more wild chickens or cows and very few wild pigs.

BENZIE: If you aware of the potential impacts, presumably it would be of interest to you to know what

they might be. Is it possible for us to get some material from you to include in our population genetics studies?

HESLINGA: We can talk about that. Palau was not included in the ACIAR project initially.

J. MUNRO: How does the survival of *T. gigas* compare with that of *T. derasa* in your experience?

HESLINGA: We have a study funded by the National Marine Fisheries Service called the Regional Yield Trials of Commercially Valuable Giant Clams. In year one we looked at locations in Palau and Western Samoa and several points in between. Side by side replicated trials showed that starting with seed clams approximately one year old, *T. derasa* has superior growth and survival in five out of six cases. We're writing up those results and in year two *Hippopus hippopus* will be compared with *T. derasa*, but we know that *Hippopus* is very hardy. In year three we will look at *T. squamosa* and *T. derasa*. Our intention is to let people decide what's best for them in their own backyard.

J. MUNRO: It seems that the survival rates of *T. gigas* in the Solomon Islands are lower than on the GBR - although predation seems to be a worse problem in the Solomons than on the GBR.

HESLINGA: We found that in the very first batch of *T. gigas* that we ever produced. We raised them side by side with *T. derasa*, and there was no contest - *T. gigas* just did not survive. Of course it depends on the location of the farms.

Philippines, 1

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MINGOA-LICUANAN, S. 1993. Philippines (local report 1), p. 40-43. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

Status of Stocks. A survey of stocks was made in 1984-1986 in conjunction with Silliman University. A total of 477 transects were made mainly in Luzon and the Visayas. The results are shown in Table 1, and Fig. 1 shows where the survey sites are located. Broodstock for the smaller species are still to be found in quite large numbers in some areas, e.g., *T. maxima* in Cagayan, and *T. crocea* in Polillo and Palawan, but not for *T. gigas* or *Hippopus spp.* Harvesting of *T. crocea* is now taking place in Polillo, as the 1985 CITES ban has been experimentally lifted by the Bureau of Fisheries and Agricultural Research for this species.

Facilities for Rearing. At Bolinao, where UPMSI has its field station, we have the following facilities: 75,200 l of tank space for larval rearing, 147.4 m^2 of tank space for settlement and juvenile rearing, 3 ocean nursery sites totalling 1,024 m^2.

The number of broodstock clams are given in Table 2. We have successfully spawned five species at Bolinao: *T. derasa* April 1989; *T. squamosa* April 1986; *T. maxima* February 1987, April 1987, February 1992; *T. crocea* March 1992; *H. hippopus* February 1987, February 1992.

There seems to be spawning seasonality, which varies with species. For instance, *T. derasa* may spawn early in summer, while *T. maxima* seems to spawn throughout the year in the Philippines.

Re-establishment of Stocks. We have deployed giant clams to various sites in the Philippines in response to requests from individuals or institutions as shown in Table 3. We would wish to visit these sites every 3-6 months, but that is expensive, and our resources go into trying to improve production. We intend to concentrate on the Bolinao area and to hold seminars and to inform the local people and officials about giant clams. We would like to be able to re-establish our stocks as sustainable populations in marine sanctuaries for the benefit of future generations as well as the present.

Discussion

NEWKIRK: Putting aside the problems we heard about this morning from John Munro on restocking in terms of numbers, time scales and so on, what are the long-term objectives of a restocking program? Is it for somebody else's benefit?

MINGOA-LICUANAN: We want to have larger numbers of giant clams.

NEWKIRK: My question is: why increase those numbers?

MINGOA-LICUANAN: We have done surveys and obtained the impression that there used to be more clams in certain places and that they have been fished out.

NEWKIRK: But who is going to benefit from restocking? I assume you're talking about re-establishing the fishery.

MINGOA-LICUANAN: That's very much so in the long term. We're also trying to persuade the local people to establish certain areas where the marine resources can be kept intact; we want to conserve whatever is left. As to who will benefit, I think that the generations to come will benefit.

ABLAN: We could have giant clams in sanctuaries forming a sustainable population. Then in addition to responding to local requests from farmers for seed clams, there is the benefit to tourism and education.

LINDSAY: Do you think you can stop theft of giant clams?

ABLAN: We can try.

Table 1. Summary of population densities in localities surveyed by the UPMSI and the SUML(*) in 1984-1986.(Tc=*Tridacna crocea*, Tm=*T. maxima*, Ts=*T. squamosa*, Td=*T. derasa*, Tg=*T. gigas*, Hh=*Hippopus hippopus*, Hp=*H. porcellanus*).

	Species density (clams/ha)							
Locality	Tc	Tm	Ts	Td	Tg	Hh	Hp	Total
Luzon								
W Pangasin	7.4	1.1	3.2					11.7
Zambales	18.3	6.7	1.0					25.9
Calatagan	12.6	10.8	26.1					49.5
Lubang	56.4	14.8	13.4			0.7		8.2
Ambil	26.8	44.8	82.0	9.0	1.0	2.0		109.2
Apo Reef	29.5	94.3	1.1	1.1				126.1
Puerto Gal.	2.7	9.6	9.6					21.9
Albay	82.1	70.3	41.4					193.7
Sorsogon	31.1	81.8	2.7					115.5
Polillo	3,399	53.3	70.0	2.9	1.0	2.4		3,528.5
Visayas								
C Visayas*	16.3	8.0	6.7					31.0
W Visayas*	22.9	30.0	131.4					184.3
NE Negros	6.9	3.4	3.4					13.7
Palawan								
El Nido	109.8	9.0	49.0	4.7		4.7	0.4	167.8
In-Aborlan	1.0	6.7	2.2	2.2				11.1
Sombrero Is.	250.0	65.0	10.0	5.0				330.0
Cagayan Is.	51.6	260.9	4.7		1.6	7.8		326.5
Cayagan*	180.7	255.1	12.4					448.2
Palawan*	3,286.2	26.7	27.1	3.8		13.8		3,357.6
Mindanao								
Camiguin Is.	11.3	31.0	15.5					57.7
Punta Sulaoan								0.0

Table 2. Numbers and source of broodstock held at Bolinao, June 1992.

Species	Wildstock	Cultured	Total	No. and source
T. gigas	1	105	106	1 JCU
T. derasa	43	138	181	4 MMDC 1 SUML
T. squamosa	61	3	64	1 MSI
T. maxima	13	71	84	2 MSI
T. crocea	77	-	77	
H. hippopus	60	297	357	2 SUML 1 MSI
H. porcellanus	4	9	13	2 SUML
TOTAL	259	623	882	

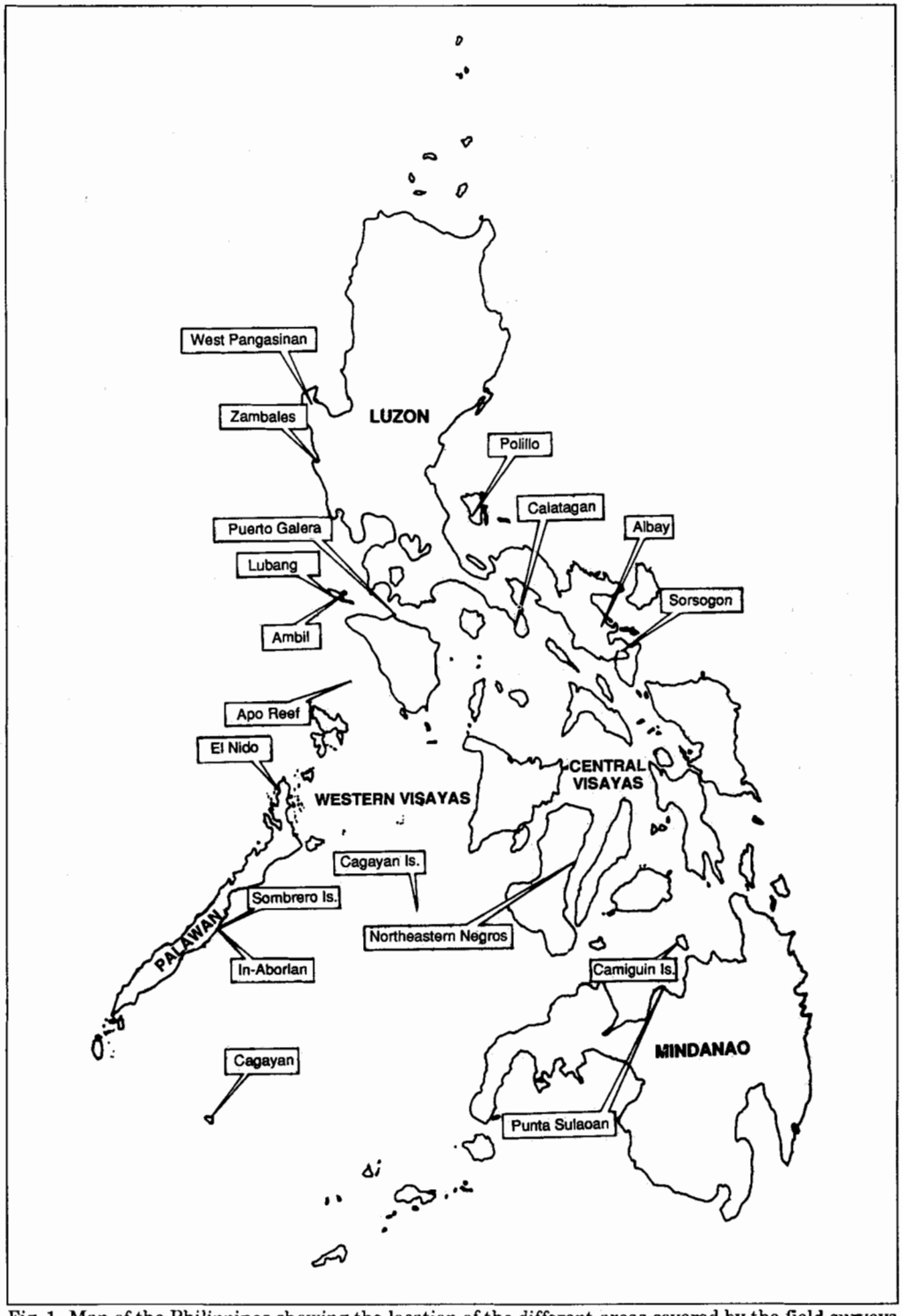

Fig. 1. Map of the Philippines showing the location of the different areas covered by the field surveys carried out in 1984-1986.

Table 3. Survival of re-established stocks of giant clams in the Philippines at six different sites in 1991-1992.

Place and date observed	Species date deployed and source	No. and approx. size (cm) when deployed	No. surviving	% Survival
Masinloc				
Nov. '91	Hh 2/87 UPMSI	60 (17 cm)	24	40
	Td 12/85 MMDC	37 (18 cm)	9	24
	Tg 10/85 JCU	4 (39 cm)	1	25
	TOTAL	101	34	
Puerta Galera				
Jan. '92	Td 12/85 MMDC	24 (20 cm)	23	99
	Hh 2/87 UPMSI	72 (16 cm)	51	71
	Tm 2/87 UPMSI	12 (12 cm)	12	100
	Tg 3/90 CAC	25 (10 cm)	24	99
	TOTAL	133	110	
Hundred Islands				
July '92	Td 12/85 MMDC	23 (22 cm)	18	78
	Hh 2/87 UPMSI	80 (18 cm)	75	94
	Tg 3/90 CAC	48 (16 cm)	44	92
	TOTAL	151	137	
Tawi-Tawi				
Dec. 91	Tg 3/90 CAC	49 (99 cm)		
	Tg 2/91 CAC	100 (33 cm)		
	TOTAL	149		
SUML	Tg 10/85 JCU	100	3	3
	Tg 3/90 CAC	100	0	0
	Tg 12/90 JCU	100	88	88
	TOTAL	300	91	
Scarborough				
May '91	Td 4/89UPMSI	25 (8 cm)		
	Hh 8/85 SUML	25 (14 cm)		
TOTAL		50		

Philippines, 2

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CALUMPONG, H. 1993. Philippines (local report 2), p. 43-55. *In* P. Munro (ed.) Genetic aspects of conservation and cultivation of giant clams. ICLARM Conf. Proc. 39, 47 p.

The goals of the SUML giant clam hatchery are:

1. to produce spat for a.) farming (*T. crocea, T. maxima, T. squamosa* and *H. hippopus*); b.) restocking reefs (*T. gigas, T. derasa* and *H. porcellanus*);
2. to train prospective hatchery owners and farmers;
3. to encourage community-based marine resource conservation using giant clams;
4. to conduct research.

The spat produced for farms is distributed by government agencies. The outlook is not good because of the very low survival rates obtained by the farmers. There are also three private companies interested in commercial giant clam farming at present.

In the case of restocking, resorts are interested in getting spat, especially of the large and colorful species. The purpose of our restocking program is to establish effective breeding populations, which can be left on the reef. Table 1 shows the numbers and sizes of clams that we have placed at various sites in the Philippines. We shall try to continue to monitor these areas.

We have found that rearing giant clams is a very effective way of encouraging community-based marine conservation. We have had a lot of success in this, especially among school children. The hatchery is like a zoo for them, where they can touch the giant clams.

Broodstock and Facilities at SUML. The broodstock available to us at Silliman are given in Table 2. The facilities consist of: 3 hatching tanks of

Table 1. Number and sizes of giant clams in restocked areas of the Philippines, June 1992

Site	Species	No.	Age (years)	Mean SL	Parentage
Apo Is. Marine Park	*T. squamosa*	33	5.5	14 cm	Carbin Reef
	H. porcellanus	16			
Pamitican	*H. hippopus*	9	6.9	15.5 cm	Manjuyod
Cabulutan Tatasan	*T. squamosa*	14	4	11 cm	Carbin Reef
Tinaogan Bindong	*T. squamosa*	19	2.4	11.4 cm	Carbin Reef
Bolinao	*T. derasa*	34	5	>15 cm	Palawan
	H. porcellanus	5	6	>15 cm	Palawan
		4	4		Palawan
	H. hippopus	10	7		Manjuyod
		24	7		Manjuyod

Table 2. Potential broodstock at SUML in June 1992. (w = wild, F1 = first filial generation, u = unknown).

Species		Number	Age (years)	Size range (cm)	Sources
T. gigas	w	9	u	31.6-58	Selinog Is., N. Mindanao Quiniluban & Cagayan Is.
	F1	3	4	32.6-36.1	JCU
T. derasa	w	8	u	27.9-38.7	Quiniluban & Cagayan Is. Palawan
	F1	3	5	16.9-21.8	Palawan x Palawan
	F1	90	4	90-112	Cagayan Is. x Cagayan Is.
	F1	1	7.6	22	Palau
T. squamosa	w	48	u	17-33	Quiniluban, Palawan
	F1	40	5	9-13	Carbin Reef x Carbin Reef
T. maxima	w	4	u	11-20	Bantayan, Dgte. City
T. crocea	w	3	u	9-10	Sibulan, Negros Oriental, Bantayan, Dgte. City
H. porcellanus	w	3	u	17-28	Cagayan Is., Palawan
	F1	108	6	10-17	Palawan x Palawan
H. hippopus	w	94	u	12-29	Pamalikan Is., Palawan, Manjuyod, Negros Or.
TOTAL	w	169			
	F1	245			

Table 3. Counts of giant clam species in Marine Sanctuaries April-May 1992.

Site	Species	No. per 500 m²	Range of SL (in cm)
Apo Is. (Negros)	*T. crocea*	1	5 - 7
	T. maxima	2	8 - 10
Sumilon Is. (Cebu)	*T. crocea*	7	2.9 - 13
	T. maxima	1-2	9 - 13.7
	T. squamosa	1	15
Balicasag Is. (Bohol)	*T. crocea*	12	2.5 - 9.6
Pamilican Is. (Bohol)	*T. crocea*	23	2.6 - 10.2
	T. maxima	5	14.7 - 16.3
	T. squamosa	22	16 - 29.5
N. Tubbataha (Palawan)	*T. crocea*	15	2.3 - 11.2
	T. maxima	4	11.3 - 22.2
S. Tubbataha	*T. crocea*	22	4.2 - 11.1
	T. maxima	2	8.4 - 9.3

10,000-l capacity each - stocked at 8.5 million/600 l; 6 larval tanks of 30,000-l capacity each - stocked at 2 million veligers per tank; 15 rearing tanks, rectangular, of 15,000-l capacity each - stocked at 5-10 juveniles/tank.

Our target output is 4000 juveniles/month of 3-4 cm SL. We can sell *T. crocea* of that size for 5 Pesos each. Presently we are getting 10% survival, and we hope to improve that to 40%. In the field the survival is around 5%. We attribute 50% of the mortality to storms, typhoons etc., about 30% to predation, about 9% to poachers and about 3% to transport and handling problems.

Table 3 gives the results of a survey of numbers of clam in sanctuaries at various sites. These sanctuaries are protected areas in which people are fined if they are caught fishing. The enforcement varies from area to area.

Discussion

HESLINGA: What are the prices you get for the *T. crocea*?

CALUMPONG: We are selling to the government 3-4 cm *T. crocea* for about 20 US cents. We found that when we sell *T. crocea*, we have to include the substrate, because in farming trials the clams died after removal from their substrate. *Hippopus* and *T. derasa* were alright after removal from their substrate. We have had many *Hippopus* die but we think this is not due to substrate removal, but due to boring sponges.

Fiji

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Background/Summary. Giant clams are traditionally a favored seafood of the Fijian people. Four species, *T. derasa*, *T. squamosa*, *T. maxima* and *T. tevoroa*, are found in Fiji. *H. hippopus* is found in the fossil records and *T. gigas* is believed to have become extinct in the last two decades. Clams occur within areas of customary fishing rights, and they are often reserved for special occasions or kept aside as a reserve food source in difficult times such as during poor fishing.

Giant clams used to be relatively abundant in reefs around Fiji, but by the late 1970s the Fisheries Division was concerned that stocks were becoming depleted because of commercial harvesting of clams due to local demand, and increasing foreign interest. Fiji recognized the need to assess giant clam resources and to monitor exploitation.

A project proposal was put to ACIAR seeking financial assistance, and Fiji was accepted as a partner in the ACIAR-funded project on "The Culture of Giant

Clam for Food and Restocking of Tropical Reefs" from 1984 to 1992. This support has enabled Fiji Fisheries Department to implement two phases of a three-phase development project.

Phase 1 involved a survey of natural populations of giant clams of all species in Fiji as well as providing information on growth rates, population structure, natural habitat and abundance. The survey provided the Fiji government with the justification for placing a 10-year ban on the export of giant clam meat in 1988. This ban was to prevent decimation of natural stocks mainly driven by lucrative export markets in Taiwan, and to preserve a core population for regeneration of the *T. derasa* stocks. Several reefs with former high population density were found to be almost completely denuded of *T. derasa*.

Results of the Fiji Fisheries Division surveys (Adams et. al 1988) showed that natural recruitment appeared to be very low. Some reefs in the Lau group are densely populated with *T. derasa* and *T. squamosa* adults but juveniles are rarely seen.

Broodstock. *T. derasa* is the main species cultured on Makogai island. Parent stock were collected from nearby islands, Wakaya, Naivai, Kovo, Batiki, and about 30 broodstock were collected from Lau group (about 180 km away). We have more than 200 *T. derasa* broodstock, 60 *T. squamosa* and *T. maxima* which were collected from the wild population. One hundred and fifty *T. gigas* broodstock (7 years old) were imported from James Cook University in 1986, most of which have attained an average shell length of about 37 cm. Fifteen thousand *H. hippopus* were imported last year, and will be used as our future broodstock.

Hatchery/Nursery. The facility on Makogai Island has been producing over 100,000 seed clams per year for the last two years, mainly *T. derasa*, and also *T. squamosa*. The Makogai ocean nursery, directly in front of the hatchery, consists of more than 2,000 1-m^2 concrete and chicken wire cages, with each cage containing up to 200 clams.

As well as testing giant clam protocols suitable for use in the Fiji rural situation, we have made several trial placements totalling about 1,000 clams in Bega, Mamanutha group, Lau and Quiva under the supervision of resort owners and selected village elders.

Extension Program. Phase 3 is intended to be the extension of giant clam farming/restocking techniques to rural and island situations. The Makogai hatchery would become a production unit rather than an experimental one, and project staff would perform training, both in the hatchery and on site. Makogai has been involved in several training activities, including regular courses attended by students of the University of the South Pacific; and training courses for Fisheries Extension Staff, villagers and Regional Officers.

Marketing. Markets will be explored using the production from Makogai ocean nursery, but the initial aim of the extension exercise is to encourage village-supervised restocking for the purpose of occasional traditional and subsistence use. It is envisaged that the village level activity would be fully subsidized during phase 3, which is expected to run for two years. Commercial operations (resort owners, hotels) will be able to purchase seed clams and supply their own growout cages. Trial shipments will be made to Japan and other Asian countries.

Funding. A multilateral project on giant clam mariculture has been proposed to AIDAB (Australian International Development Assistance Bureau). Minimal funding would be available from the AIDAB project for operational costs. There is a high possibility that Fiji government will provide financial support for the next 5-10 years while the farming techniques are being fine-tuned, and while the farm is being developed to become a commercially viable operation. Japan International Cooperation Agency (JICA) has been approached for assistance and it also has shown interest.

Future Plans. 1) Mass production of giant clams with an annual hatchery production of 15,000 juveniles at 6 months of age. Of these 50,000-75,000 clams will be given to villages for restocking of reefs, and the remainder will stay at Makogai ocean nursery to expand our production. This farm is planned to be self-supporting in five years with clams being marketed locally and possibly overseas. 2) Further research is required over the next few years, to develop the most suitable methods for the ocean nursery and growout phases under Fijian environmental conditions. This will include the maintenance procedures required and continued investigations on how to control predators. Studies on developing the most reliable and cost-effective protective cages for juvenile clams should continue. 3) Training courses for villagers to continue. 4) The development of Makogai as a marine research center for the Fiji Fisheries Department and for research in related fields will be encouraged, designed in a way so that other research programs do not interfere with clam seed production. 5) Public education programs will be conducted to make the public aware of the decline in clam abundance, and that overfishing could result in the extinction of these species from Fiji, as has already happened with *T. gigas*.

Conclusion. Giant clam genetics has not been a major topic in giant clam research. Fiji Fisheries Department would very much like to cooperate with geneticists who are here today and should a collaborative study program be implemented in the near future, Fiji Fisheries is willing to participate.

References

Adams, T.J.H., A.D. Lewis and E. Ledua. 1988. Natural population dynamics of *Tridacna derasa* in relation to reef re-seeding and mariculture, p. 78-81. *In* J.W. Copland and J.S. Lucas (eds.) Giant clams in Asia and the Pacific. ACIAR Monograph 9, 274 p. Australian Centre for International Agricultural Research, Canberra.

Discussion

GOMEZ: How many *T. tevoroa* do you now have and how are they doing?

LEDUA: I took 500 juveniles from Tonga to Makogai and they are still in our tanks. We have had 2% mortality up to now. Last week our staff managed to locate one *T. tevoroa* at Makogai and it's now in our ocean nursery.

CALUMPONG: The juveniles that you are going to give to the villagers to farm - what conditions do you stipulate?

LEDUA: That they try to raise them and put them on the reef. We ask them to try to keep a good stock for about seven years and then they can sell them. On management we leave it to the people. We advise them that the stocks are running out and they should conserve them.

J. MUNRO: What size are they when they go out on the reefs, and are they in nursery cages?

LEDUA: Three cm. The villagers provide their own cages.

ALCAZAR: Have you any *T. derasa* juveniles from your own stock?

LEDUA: We have more than 200,000 juveniles of *T. derasa*.

GERVIS: Is there any strategy in place for the clams you send to the villages? Do you send them from one cohort, or do you try to mix different cohorts?

LEDUA: We produce 150,000 juveniles each year and send 50,000-75,000 to villages. The rest we keep at Makogai for us to work on.