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## MOLLUSCAN AND CRUSTACEAN GENETIC AND BIOTECHNOLOGY INTERVENTIONS: A REVIEW

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### ABSTRACT

*Global demand for seafood products alone is projected to increase by 70 percent in the next 30 years as harvests from traditional capture fisheries either remain stable or continue to decline. At the same time, coastal marine ecosystems worldwide that support wild shellfisheries are threatened by pollution, habitat degradation, overharvesting and a growing dependence on common pool-resources among other concerns, lending an increasingly important role for sustainably produced and managed shellfish resources to fill the widening gap in the world's capacity for food production, hence the application of genetic technology. This paper then review the importance of genetics application in shellfisheries like species identification, stock structure, mixed stock analysis, biomarkers for age, harvest rate and abundance, genetic diversity, detection of pathogens and invasive species among others. Various methods of genetic approach, challenges and strategies for promoting these genetic methods in Nigeria were also presented.*

**Keywords:** Shellfish, Genetics, Economically importance, Mollusca, Crustacean, Nigeria

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### INTRODUCTION

Shellfish, a broad description for aquatic invertebrate animals possessing a hard outer covering includes the molluscs (oysters, clams and snails) and the crustaceans (shrimps, prawns, lobsters and crabs) (Yeo *et al.*, 2008). Management of shellfish populations is becoming an increasingly common practice worldwide, as natural fisheries succumb to pressures of overharvesting, habitat loss or degradation, challenges from invasive competitors and pathogens (Wenne *et al.*, 2007). Shellfish management is concern with changes in effective population size of the wild population, and changes in the genetic composition of the recipient population. These changes may affect the ecological balance of the habitat if not well managed. Perpetuation of the resource is the common goal of all shellfish management programs. Genetic factors affect this goal because shellfish are the products of their genes, the environment and interactions

between the two. The genetics of shellfish, in connection with the environment, determine the quality and persistence of a shellfish resource (Gaffney, 2006).

Shellfish managers must realize that implementation of regulations, stocking strategies and other management activities affect the genetic make-up of shellfish stocks. According to Kapuscinski and Miller (2007), management activities that influence the genetics of shellfish stocks include: (i) maintenance of shellfish species with adequate natural reproduction, (ii) regulation of shellfish population through size or gear restrictions, (iii) enhancement of a shellfish population with marginal natural reproduction and (iv) rehabilitation of depleted shellfish resources by stocking or control of harvest.

Often, managers have concentrated on manipulation of non-genetic, environmental aspects of shellfisheries like harvest control, stocking, and pollution abatement.

There are at least two problems with this approach. First, a primary determinant of the quality of shellfish resources (genetics) has been neglected entirely (Gaffney, 1996). Second, management activities inadvertently affect the genetic make-up of shellfish stocks. Management costs due to the effect of management activities on the genetics of shellfish stocks have not been adequately considered (Purcell *et al.*, 2006). This last point is particularly unfortunate because relatively small and inexpensive changes in management practices may affect substantial improvements in the genetic integrity of a stock (Reynolds *et al.*, 2005).

Many shellfish populations have been over exploited or reduced by changes in local environments. These populations are endangered and some indigenous populations are already extinct and hence there is an urgent need for knowledge on their basic population structure (Dulvy *et al.*, 2003; Moruf and Ojetayo, 2017). Shellfish management and species restoration efforts are now enhanced through application of conservation genetic analysis techniques. The field of genetic technology similarly ranges from simple techniques such as hybridization, to more complex processes such as the transfer of specific genes between species to create genetically modified organisms (Avisé *et al.*, 2002). The purpose of this review is to summarize recent applications of genetics in shellfisheries, the various methods of genetic approach, challenges and strategies for promoting these genetic methods in Nigeria.

## MATERIAL AND METHODS

A comprehensive internet search of literature on molluscan and crustacean genetic and biotechnological interventions was undertaken using Google Search and other search engines. Literatures obtained were analyzed in pros and relevant cited tables adopted. Figures 1 – 11 are photographs of shellfishes from Nigerian coastal waters provided by the first author.

## RESULTS

### Biology Underlying the Genetics of Shellfish

Shellfish, in fisheries term is an edible aquatic invertebrate with hard outer covering. In Nigeria, some economically important edible molluscs (Figures 1 – 7) and crustaceans (Figures 8 – 11) constitute cheap source of animal protein and their shells provide a source of calcium in animal feed (Moruf and Lawal-Are, 2015).



**Figure 1: *Crassostrea gasar* (Ostreidae). Site of Collection: Lagos Harbour, Nigeria (6°44'19"N and 3°40'47"E)**



**Figure 2: *Egeria radiata* (Donacidae). Site of Collection: Forcados River, Delta State, Nigeria (N05°18'31" and E06°10'27")**



**Figure 3: *Anadara senilis* (Arcidae). Site of Collection: Upper Bonny River estuary in the Niger Delta, Nigeria (N4°47' and E7°15')**



Figure 4: *Mytilus edulis* (Mytilidae). Site of Collection: Ofiki River, Oyo State, Nigeria (N07°25' and E03°7')



Figure 8: *Panulirus regius* (Palinuridae). Site of Collection: Off Nigeria Coast (N6°39'56" and E3°39'37")



Figure 5: *Thais haemastoma* (Muricidae). Site of Collection: Takwa Bay, Lagos State, Nigeria (N05° 40' and E07° 09')



Figure 9: *Macrobrachium macrobrachion* (Palaemonidae). Site of Collection: Lagos Lagoon, Nigeria (N6° 26' and E3° 50'E)



Figure 6: *Tympanotonus fuscatus* (Potamididae). Site of Collection: Abule-Agege Creek, Lagos Lagoon, Nigeria (N6° 26' and E3° 23')



Figure 10: *Penaeus monodon* (Penaeidae). Site of Collection: Lagos Lagoon, Nigeria (N6° 26' and E3° 50'E)



Figure 7: *Sepia officinalis* (Sepiidae). Site of Collection: Off Nigeria Coast (N6°39'56" and E3°39'37")



Figure 11: *Portunus validus* (Portunidae). Site of Collection: Off Nigeria Coast (N6°39'56" and E3°39'37")

The genetics of individual shellfish is the foundation for the genetics of broodstocks and populations (Kapusinski and Miller, 2007). The attribute of interest may be a qualitative trait or a quantitative trait. A qualitative trait can be described without measurement (e.g. sex), while quantitative traits are described by a count or measurement (e.g., Girth size in periwinkle, total weight of a shellfish). Shellfish, unlike most terrestrial animals, can live with extra sets of chromosomes (polyploidy) or with additions or deletions of individual chromosomes (aneuploidy), both in somatic tissues and in the germ line (Kapusinski and Miller, 2007). Triploids, having three rather than the normal two sets of chromosomes, tend to be sterile, which can be a useful property in aquaculture of native and non-native shellfish species (Suttle, 2005). According to Shumway and Kraeuter (2000) the biology underlying the genetics of molluscan shellfish includes: (i) many molluscan shellfish show evidence for substantial hybrid vigor in growth or yield, (ii) crossbreeding, in addition to selection, is likely to be important in improving the yield of farmed molluscan shellfish, (iii) Pacific oysters and, by inference, many bivalve molluscs, carry unusually large loads of lethal mutations, which cause substantial deviations from the normal rules of inheritance and probably explain hybrid vigor in growth or yield observed upon crossing of inbred lines and (iv) sex determination in many molluscan shellfish is much less fixed than in most terrestrial animals. Individuals can change sex through their lives or can even be both sexes simultaneously (hermaphrodites). This has practical implications in shellfish breeding programs, if sexes differ in performance or productivity. Females of the Pacific oyster, for example, are about 15 % heavier than their male siblings at harvest.

### **Adaptive Genetics and Shellfish Research**

The development of DNA-based genetic techniques has had a revolutionary effect on management of many shellfish species. Genetic approaches have and are being used to examine many different questions of importance to this industry and of particular focus has been the

identification and use of adaptive markers. There is evidence of local adaptation in many species of fish and shellfish and locally adapted populations are often characterized by heritable traits allowing them to survive and thrive under heterogeneous environmental conditions (Sanford and Kelly, 2011). Some such traits are often of particular interest in relation to aquaculture production (e.g. those associated with growth rates, survival to biotic or abiotic factors).

The fast developing field of genomics offers a potential to identify the necessary markers linked to such adaptive traits (Stinchcombe and Hoekstra, 2007). Once identified, these markers can be utilized in marker assisted selection (MAS) programmes which seek to identify individuals exhibiting beneficial genetic variants and using these as broodstock to achieve enhancement of the selected line within the aquaculture environment (Ribaut and Hoisington, 1998). Identification of such adaptive markers relies on techniques which link genetic variation to heritable phenotypic variation at the traits of interest. This involves genome scans using one of a number of genetic markers of a number of individuals which differ in the trait of interest. Various statistical approaches can then be used to identify those markers linked to the trait and if data are available to position this marker on the genome of the species (Lynch and Walsh, 1998). In theory therefore it might be expected that the same or similar techniques could be used to identify and utilize such markers in the shellfish aquaculture industry.

However, there are a number of characteristics of shellfish species which have to be taken into account when performing such investigations in these species and which may mean that different approaches may have to be used than those typically employed with finfish. First, and perhaps most importantly, many shellfish aquaculture species still rely on collection of individuals from the wild for growing on (i.e. seed collection) (Hold *et al.*, 2012). In many of these species therefore there will be no selected lines to work with unlike in the finfish context.

Selective improvement of lines using techniques such as MAS cannot be envisaged. This does not mean however that both the identification and use of adaptive markers is also impossible. Rather than using selected lines, as is often the case in the finfish context, wild individuals which still differ in the trait of interest can be examined, genotyped and adaptive markers identified (Kapuscinski and Miller, 2007). Particular populations of individuals which show beneficial phenotypes can then be targeted for the collection of individuals for growing on with such populations being identified using the available adaptive markers.

Furthermore, the particular life history characteristics of many shellfish species means that development of this local adaptation that may be utilized in such stock selection programmes may be different from that seen with many finfish species (Moruf and Lawal-Are, 2017a). Many bivalves exhibit only weak genetic structure, or even panmixia, when examined using neutral markers which indicates significant gene flow between populations and which may suggest a lack of local adaptation (Riginos and Cunningham, 2005). Furthermore, the high fecundity, broadcast spawning and pelagic larval phases might all act to prevent local adaptive selection from occurring (Riginos and Cunningham, 2005). However, it should also be noted that the often large shellfish populations have substantial within population genetic variation which provides opportunities for natural selection in different ecological settings and thus has the potential to result in local adaptation. Such adaptation has already been reported in a number of invertebrate species of interest to the aquaculture industry. For example, Pespeni *et al.* (2012) reported significant differentiation at functional genes between populations of the purple sea urchin (*Strongylocentrotus purpuratus*). Riginos and Cunningham (2005) showed strong evidence of local adaptation in the *Mytilus* species complex even at small spatial scales. Yanick *et al.* (2003) and Sanford and Worth (2010) showed local adaptation in the snail *Nucella canaliculata* using reciprocal translocation experiments.

A number of molecular markers have been used in shellfish research; microsatellites have often become the marker of choice for application in fish and shellfish population genetic studies (Cruz *et al.*, 2005). They have multiple alleles which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana-Eguia *et al.*, 2004). Microsatellites are preferable because they are potentially codominant and highly polymorphic. In addition these markers have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations (Brinez *et al.*, 2011).

The choice of marker for a particular application will vary and depend on a number of variables including the availability of the marker type in the species under investigation, how polymorphic is the marker, the technology available, the speed of genotyping, the ease and accuracy of scoring, the degree of genome coverage required, the level of discriminatory power required and of course cost. Table 1 summarized some of the techniques often used to examine issues in the shellfish research, while Table 2 makes some suggestions as to possible marker types that may be of most use.

### **Current Application of Genetic Biotechnology in Shellfish Research**

The use of genetic technologies in shellfish research is very limited and this is represented in a few fields such as genetic improvement, reproduction and disease control/management.

#### **A. Genetic improvement**

**Polyploidy:** This is a genetic manipulation by increasing the number of sets of chromosomes. Many shellfish species are relatively tolerant to chromosomal manipulation in the early stages of their development. The use of genetic manipulation including polyploidy (i.e. increasing the number of sets of chromosomes) to improve shellfish culture production has been examined.

**Table 1: Markers available for some shellfish species**

| Shellfish Species                                   | Marker types                             |
|---|--|
| <i>Crassostrea gigas</i> (Pacific oyster)           | Allozyme, AFLP, mtDNA and Microsatellite |
| <i>Crassostrea virginica</i> (Eastern oyster)       | AFLP, mtDNA, Microsatellite and SNP      |
| <i>Mytilus edulis</i> (Blue mussel)                 | AFLP, mtDNA, Microsatellite and SNP      |
| <i>M. galloprovincialis</i> (Mediterranean mussel)  | mtDNA, RFLP, Microsatellite and SNP      |
| <i>Dreissena polymorpha</i> (Freshwater mussel)     | AFLP, mtDNA, RFLP and Microsatellite     |
| <i>Ostrea edulis</i> (European flat oyster)         | Allozyme, AFLP and Microsatellite        |
| <i>Aequipecten irradians</i> (Atlantic bay scallop) | AFLP, mtDNA, RFLP and Microsatellite     |
| <i>Pecten maximus</i> (King scallop)                | mtDNA, RFLP and Microsatellite           |
| <i>Mercenaria mercenaria</i> (Hard clam)            | Allozyme and Microsatellite              |
| <i>Solen marginatus</i> (Razor clam)                | Allozyme, RFLP and Microsatellite        |
| <i>Aequipecten opercularis</i> (Queen scallop)      | Allozyme, mtDNA, RFLP and SNP            |
| <i>Venerupis senegalensis</i> (Pullet carpet shell) | RFLP and RAPD                            |

Source: ICES (2014)

**Table 2: Suggested marker systems for shellfish genetics**

| Tasks                   | Recommended marker system   | Other useful markers  |
|-------------------------|-----------------------------|-----------------------|
| Species identification  | mtDNA, SNPs                 | AFLP, allozymes       |
| Strain identification   | mtDNA, SNPs, Microsatellite | RAPD, AFLP            |
| Hybrid identification   | mtDNA, SNPs, Microsatellite | RAPD, AFLP            |
| Paternity determination | SNPs, Microsatellite        |                       |
| Genetic diversity       | mtDNA, SNPs, Microsatellite | RAPD, AFLP, allozymes |
| Genetic mapping         | SNPs                        | Microsatellites, AFLP |
| Comparative mapping     | SNPs                        | Microsatellites, RFLP |

Source: ICES (2014)

Triploids (with three sets of chromosomes) may be useful for management programmes where sterility can prevent introgression of genes from escaped individuals of commercial stocks into natural populations, or in commercial operations where sterile fish are desirable to prevent side effects such as deterioration of carcass quality due to maturation or the occurrence of high mortalities in stocks when males mature early or that occur prior to maturation.

**Genomics:** This includes the intensive efforts to determine the entire DNA sequence of organisms via fine-scale genetic mapping. One of the major constraints in the rearing of many different shellfish species is the lack of adequate genomic information.

**Genetic modification:** A genetically modified organism (GMO) is one whose genetic material has been altered through genetic engineering techniques with DNA molecules from different sources that are combined into one molecule to create a new set of genes. Typically, it involves introduction of a single gene from an unrelated

species. After about two decades of very intensive research, the technology has reached the stage where it is possible to produce GM carp, tilapia and salmon (Martinez, 2007). However, no aquatic GMOs have yet been developed for shellfish species and many developing countries have yet to develop a clear policy on the use of transgenic fish.

## B. Control of reproduction

**Gynogenesis/Androgenesis:** Gynogenesis is the production of an embryo from an egg after penetration by a spermatozoon that does not contribute genetic material. Androgenesis is the production of an embryo from an egg whose DNA was inactivated and which was fertilized using normal sperm. In both cases, the diploidy is restored using heat/cold shocks. In gynogenesis, if diploidy is restored soon after fertilization, the procedure is called meiotic gynogenesis due to the fact that the second polar body is retained, and this procedure is similar to what is expected under autofertilization in terms of inbreeding. If shocks are applied later or in androgenesis where the

ova were DNA-irradiated for DNA inactivation, the same chromosome is duplicated and thus the embryo is a double haploid individual which is completely inbred for every locus.

**Development of monosex populations:** The negative relationship between growth rate and gonadal development has been found in many shellfish species (Lawal-Are *et al.*, 2012; Moruf and Lawal-Are, 2017b). The negative side-effects of early reproductive onset that decrease the growth rate through a series of physiological mechanisms. The faster growth rate of the other sex is probably caused by its later maturation. One explanation of this finding is the appearance and accumulation of sex hormones that act as growth inhibitory agents. The advantages of monosex culture depend on the species involved (FAO, 1997). This is because one sex may be superior in growth or have a more desirable meat quality, or to prevent reproduction during grow-out or the appearance of sexual/territorial behavior (aggressiveness) that occurs when a mixed sexed group triggers the reproductive season. In many shellfish species, sex is not permanently defined genetically and can be altered by a number of factors including hormonal treatment during the early stages of development. Gonadal development starts from primordial germ cells, with females starting differentiation prior to males (Lawal-Are, 2010). The point in time when differentiation occurs depends on the species involved.

**Cryopreservation:** Is a technique which involves long-term preservation and storage of biological material at a very low temperature usually at  $-196^{\circ}\text{C}$ , the temperature of liquid nitrogen. Sperm cryopreservation has been successfully implemented for a number of cultured shellfish species, and modest success has been achieved in the cryopreservation of shellfish embryos and early larvae (Nai-Hsien and Chiu Liao, 2001). However, it is still uncertain whether this method is economically advantageous compared with disseminating improved broodstock using larval material.

### C. Pathogen screening and disease diagnostics

The control of disease outbreaks relies heavily on having rapid and accurate diagnostic tools available in order to detect and identify the pathogen causing the morbidity and mortality. DNA and RNA methods have been used extensively for detecting a number of viral and bacterial pathogens in aquaculture worldwide (OIE, 2003). The techniques rely upon the fact that each pathogenic species carries a unique DNA or RNA sequence that can be used for identification. The techniques offer high sensitivity and specificity, and the commercial development of PCR primers and diagnostic kits allows rapid screening of serious viral and bacterial infections and has direct application. Molecular-based techniques such as PCR also have applications in situations where the animal shows no antibody response after infection. For example, as molluscs do not produce antibodies, antibody-based diagnostic tests have limited application to pathogen detection in these species. These tools include both immunoassay- and DNA-based diagnostic methods, e.g. fluorescent antibody tests, enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), in situ hybridization (ISH), dot blot hybridization and PCR amplification techniques (FAO, 1999).

### Importance of Genetic Biotechnology in Shellfish Management

**Species identification:** Stock assessments typically assume that the unit under consideration is demographically homogeneous, stock identification is a necessary precursor to scientifically based shellfish management (Cadrin *et al.*, 2005). An organism's identity is fundamental to the monitoring, marketing and study of shellfish species. DNA analysis is a rapid, universal and highly accurate tool for assigning a specimen to a species, important when the specimen cannot be identified in any other way; and often the only way to identify cryptic species (Ovenden *et al.*, 2015).

**Stock structure:** Genetics detects enduring patterns of stock structure in contrast to other methods that use characteristics acquired during the lifetime of an individual e.g. parasite load, chemical composition of otoliths (Ovenden *et al.*, 2015). Fisheries managers use stock boundaries to assign quotas, model alternative harvesting scenarios and to design monitoring programs.

**Mixed stock analysis:** Naturally occurring genetic marks have some inherent advantages for the analysis of mixed-stock fisheries compared to more traditional methods: they do not require large efforts to manually mark individuals every generation; genetic markers are temporally stable compared to some other stock identification methods (such as scale pattern analysis) that need to be surveyed in baseline populations every year (Cadrin *et al.*, 2005). Genetic tools determine the proportion of harvested aggregations belonging to distinct stocks

**Biomarkers for age:** Indexing the age of specimens without otoliths. Growth estimates are essential for devising harvest strategies. The age of individuals of some shellfish species can be estimated by counting growth rings in sectioned hard parts like otoliths in finfish. Many shellfish species (e.g. crustaceans) don't have hard parts for sectioning. Counting growth rings is lethal, whereas biomarkers can be assayed from tissue taken non-lethally (Ovenden *et al.*, 2015).

**Parentage analysis:** Molecular markers can be used successfully to trace alleles inherited by progeny from a group of candidate parents, thus providing a means of parentage analysis. In many shellfish species, reproduction cannot be fully controlled and thus natural mating is the only way to produce offspring for the next generation of a breeding programme.

**Ecosystem monitoring:** Genetic tools can also provide unique understandings of ecological processes, including mapping complex food webs, measuring environmental stressors, and detecting evolutionary effects of climate change.

Harvested species including European flounder and mussels are used as biosensors for environmental contaminants (Waples and Gaggiotti, 2006).

**Harvest rate and abundance:** Abundance and other population parameters can be estimated from the recapture of individuals identified with genetic tags. Genetic tagging is a relatively new in the marine environment, but has been widely adopted for monitoring terrestrial fauna (Reynolds *et al.*, 2005).

**Genetic diversity, abundance and resilience:** Genetic diversity refers to the diversity of genetic variants in a species' gene pool. Metrics of genetic diversity can provide indications of a species' abundance and its capacity to evolve in response to environmental change (Ward *et al.*, 1994). Genetic variation is an important aspect of the genetic makeup of managed shellfish stocks. The long-term utility of genetic variation is to give populations the ability to adapt to changing environments (Ward *et al.*, 1994). The short and long-term genetic effective population size of tiger prawns in south-east Queensland were similar, implying harvest rates have been sustainable. Animal genetic diversity has provided the material for the successful breeding improvement programs of the developed world in the 19th and 20th century (Adekoya *et al.*, 2013)

**Detection of pathogens and invasive species:** Genetic assays can diagnose and quantify the incidence of diseases or invasive species in wild fisheries and aquaculture (Ovenden *et al.*, 2015).

### **Challenges and Strategies for Genetics Application in Shellfisheries**

**Environmental damage:** Transgenic techniques offer the means of producing immediate large quantum changes in performance, for example in growth rate that far exceed those attainable with other approaches. However, the exploitation of transgenic technology is hindered by legitimate concerns about possible environmental damage,

caused by escaped transgenic shellfish breeding in the wild. This fear can be allayed through the development of shellfish that are made sterile by transgenic approaches. The sterility should be reversible if so desired, that is shellfish could be made fertile by a simple treatment such as hormonal injection. This could enable brood stock reproduction and sterile fry production.

**Inadequate training and research networking with foreign partners:**

Scientists from Nigeria should be trained abroad in specific technologies in countries that have made progress in the area of shellfish genetics. Also training can be received through networking with relevant institutions across the globe. Research collaboration is a dynamic process that involves creating, mobilizing, utilizing, enhancing, upgrading and adjusting the existing capacities of individuals and local communities and institutions (Ngoile and Sarunday, 2002). Since technology transfer is a continuous process, the networking with research institutions outside the continent has to be continuous so that Nigeria will not be left behind in fish genetics technology (Erondu *et al.*, 2011).

**Lack of facilities and inadequate research fund:**

Modern equipment, facilities and more funds should be provided by the government to relevant institutions involved in research activities in shellfish genetics for the purpose of culture and conservation of shellfish resources.

**Public perception:** Public perception that use of transgenic organisms might have unpredictable and undesirable consequences is justified, in the sense that the genetic modifications made in transgenic individuals are rarely characterized. The debate on the application of biotechnology in fisheries is far from settled, as the perception of people on the issues of ethics and safety needs to be addressed. Awareness about the potential of genetics in increasing and enhancing management and production of shellfish resources should be disseminated to the fish farmers across the country.

**Conclusion:** The global rapid expansion in the farming of several shellfish species will no doubt be further boosted by application of genetic biotechnology. In developed countries, technological approaches like genetic manipulation and modification, genomics, reproduction control and disease diagnostics in many shellfish species have been successfully performed. It is on this basis that the following recommendations are suggested as ways of encouraging the use of genetic biotechnology for improving shellfish conservation and management in Nigeria. Provision of adequate modern research facilities, adequate funding of biotechnology related research, training and research networking with foreign partners and all concerned stakeholders should galvanize efforts in formulating and implementing genetic manipulation policy in a sustainable manner to ensure environmental protection, economic benefits and shellfish resource conservation.

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