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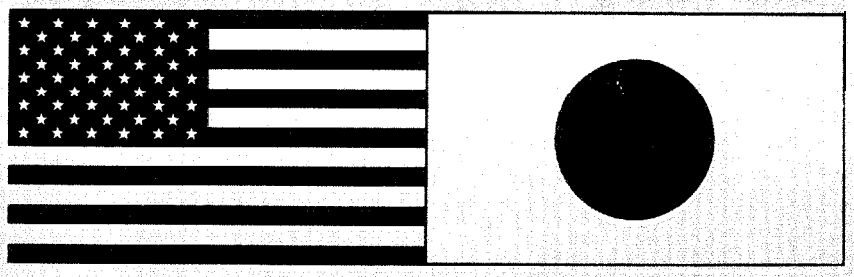
Interactions Between Cultured Species and Naturally Occurring Species in the Environment



PROCEEDINGS OF THE TWENTY-FOURTH U.S.-JAPAN AQUACULTURE PANEL SYMPOSIUM

Edited by B. Jane Keller, P. Kilho Park, James P. McVey, Kazufumi Takayanagi and Karumi Hosoya

UJNR Technical Report No. 24

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Corpus Christi, Texas, October 8-10, 1995

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Sea Grant is a partnership of university, government and industry focusing on marine research, education and advisory service. Nationally, Sea Grant began in 1966 with the passage of the Sea Grant Program and College Act. Patterned after the Land Grant Act of the1869s, the Sea Grant concept is a broad-based scientific effort to better the world for all those living in and out of the sea

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Marine Aquaculture Regulation in the United States: Environmental Policy and Management Issues

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ABSTRACT

The U.S. marine aquaculture industry is extremely young. While catfish and trout culture have existed for many decades, the cultivation of species in marine and coastal environments has only emerged within the last 30 yr. Only 15% of total domestic aquaculture production in 1991 consisted of marine species, with oysters representing 80% of marine aquaculture production. The U.S. marine aquaculture industry has not kept pace with the growth of the world industry. One major reason for this slow growth is the absence of a unified federal and state policy and regulatory framework for U.S. marine aquaculture. According to a 1983 study commission by the Joint Subcommittee on Aquaculture, as many as 11 federal agencies are directly involved in regulating aquaculture, and another 10 are indirectly involved. However, only a limited number of direct permitting and licensing requirements are imposed by federal agencies. Thirty-two state regulatory programs were examined and it was discovered that over 1,200 state laws were found to have some significant bearing on aquaculture operations. The majority of laws and regulations that specifically authorize, permit or control aquaculture are usually found at the state level. A review of the current literature suggests that neither the federal nor state regulatory situation has improved since 1983. An examination of South Carolina policy and regulatory actions was undertaken to assess the regulatory and institutional status of marine aquaculture. The state has adopted a strategic plan for aquaculture development, published an interim guidebook to aquaculture permitting, established a state aquaculture permit, and amended and passed legislation on specific industry needs. While South Carolina is viewed by many as having made significant strides in minimizing regulatory and institutional constraints, the growth of marine aquaculture in the state remains slow, and reflects the situation across the country. A number of strategies to remove the barriers marine aquaculture faces have been offered; however, without the development of an overall policy framework at the federal and state level, the potential of marine aquaculture to fulfill the country's seafood needs will remain unrealized.

INTRODUCTION

Aquaculture in the United States has the potential to become a major growth industry in the 21st century. Global seafood demand is projected to increase 70% by the year 2025 (Joint Subcommittee on Aquaculture, JSA 1993). With harvests from captive fisheries stable or in decline, aquaculture would have to increase production by 700% to a total of 77 million metric tonnes annually to meet the projected demand (JSA 1993). If there is the same type of growth in aquaculture production over the next seven yr as there has been in the last seven, by the end of the 20th century aquaculture could be supplying upwards of 25% of all the seafood consumed in the United States (Harvey 1994).

The United States currently imports more than 60% of its fish and shellfish, representing a total of $9 billion annually (JSA 1993). Seafood products are the nation's largest agricultural import, second overall to petroleum (JSA 1993). Each year, Americans consume more than $800 million of foreign-grown aquaculture products. Obviously, domestic aquaculture production has not grown at a rate necessary to offset the consumer demand for seafood.

Nevertheless, the development of the U.S. aquaculture industry is felt to be vital to the future of the nation because it promises to produce: (1) high quality seafood to replace that supplied through the harvests of wild stock in decline or at maximum sustainable yields; (2) products for export to help reduce the country's foreign trade deficit; (3) stock enhancement of important commercial and recreational fisheries species; (4) economic development opportunities for rural and suburban communities; and (5) new employment opportunites for skilled workers (National Research Council, NRC 1992).

During 1990-1991, the U.S. aquaculture industry and

its supporting services accounted for 300,000 full-time jobs with a direct and indirect economic impact of $8 billion (JSA 1993). For every additional 5 million kg of domestic aquaculture production, 1,300 additional jobs could be created on the farms and in related industries (NRC 1992).

MARINE AQUACULTURE IN THE UNITED STATES

The U.S. marine aquaculture industry is extremely young. While catfish and trout culture have existed for many decades, the cultivation of marine species has emerged only over the last 30 yr. In 1991, total domestic aquaculture production was 398 x 10^6 kg with a value of $727 million; only 15% (= 47 x 10^6 kg at $119 million) was produced by the marine aquaculture industry (Sandifer 1994). Eighty percent of the nation's marine aquaculture yield was represented by oyster production and 12% by salmon production. Forty-eight species made up the remaining 8% (NRC 1992). The U.S. marine aquaculture industry is relatively small, but it remains vital since most of the huge seafood deficit in fishery products comes from the importation of marine, not freshwater, seafood (Sandifer 1994). Marine aquaculture is now practiced in more than 80% of the states and territories of the United States. Nevertheless, cultivation of all marine species, except oysters, is in the early stages of commercial development in the United States, and most operations have yet to achieve economic stability (NBC 1992). It goes without saying that the U.S. marine aquaculture industry has not kept pace with the growth of the world industry during the East 25 yr (NBC 1992).

The future for marine aquaculture in the United States is much less certain than that of its freshwater counterpart. One serious problem is that most marine aquaculture is conducted in shallow coastal and estuarine waters, which are affected by increasing population pressures and industrial and residential pollution and development. By the year 2010.70% of the total population of the United States will live within 120 km of the coast (Culliton et al. 1990). In addition, whereas the transition from fishing to aquaculture in freshwater systems is analogous to that of hunting to farming, marine aquaculturists face an additional hurdle: the fact that they have no property interest in the "lands" they need (Nixon 1994). Because the ocean has traditionally been viewed as a common property resource, there are also conflicts with other commercial and recreational users which may slow or prevent the development of marine aquaculture (Harvey 1994).

Growth of the domestic marine aquaculture industry is dependent upon the attainment of five basic requirements (DeVoe and Mount 1989):

(1) High Water Quality Locations: The availability and maintenance of a high water quality environment is a primary need for aquaculture. The industry must be assured that current and future uses of the surrounding aquatic environment will not reduce the quality of the waters where the species are being cultured.

(2) Access to the Aquacultnre Site: In choosing sites, the industry must consider an array of environmental and operational factors. Marine aquaculture typically requires both an aquatic environment and an adjacent on-land base of operation. In choosing sites, the industry may have to obtain permission, rent, lease or purchase outright the adjacent land to assure access to the site.

(3) Assertion of Exclusive Fishing and Culturing Bights: Laws in most states provide the public with the right to use state waters for navigation, recreation and fishing. However, various methods of aquaculture used now and proposed for the future may require exclusive use of coastal or ocean waters. Such exclusive use could consequently deny to some degree the use of the area by traditional users.

(4) Financial Investment: Establishment of aquaculture operations may require a significant financial commitment; however, aquaculture as an industry is viewed by investors as a high risk activity for many reasons. The availability of funding through venture capital, public and private sector loans, or other sources will depend to a large extent on the anticipated stability of and the level of property rights to be vested with the proposed operation.

(5) Government Commitment: In the case of marine aquaculture, this requirement may be the most critical. Government must demonstrate its support by clearly defining the term aquaculture, providing supporting policy statements, offering incentives (which do not necessarily have to be solely financial) to underscore its commitment, and defining and streamlining its regulatory and legal requirements.

Furtber complicating the future of marine aquaculture is the complexity that stems from unique factors that distinguish it from other forms of agricultural activity, including: (1) the interaction of marine aquaculture with other marine and coastal activities and interests-interactions that are often characterized by conflict; (2) the fact that although marine aquaculture is ocean-based, it depends on the use of land and freshwater resources as well; and (3) the numerous environmental and regulatory considerations involved in the development and use of coastal zone land and water resources, usually held in the public trust (NRC 1992).

The purpose of this paper is to review the key institutional and regulatory issues related to marine aquaculture development in the United States, critically examine these issues in more detail through a case study analysis of the situation in the state of South Carolina, and to explore possible remedies that may alleviate constraints on and provide for a more orderly development of the marine aquaculture industry.

REGULATION OF THE MARINE AQUACULTURE INDUSTRY

CHARACTERISTICS OF THE INDUSTRY

To understand the problems that confront U.S. marine aquaculture, the basic nature of the industry must first be reviewed. Marine aquaculture represents a relatively new use of the nation's coastal resources, and it must compete for access to those resources (Nixon 1994). Newcomers to the industry, as well as local authorities, suffer from a lack of experience, in- appropriate advice on site selection, inadequate evaluation of market opportunities and product diversification, and a lack of understanding of marine aquaculture development in relation to other forms of competition (Chamberlain and Rosenthal 1995). Much of this confusion stems from its uniqueness and complexity.

Marine aquaculture requires a site of operation, including upland and water-based locations. Issues of land use and zoning, exclusive use of public lands and waters, and navigation and use conflicts must be addressed. Species cultured in a marine environment continue to raise concerns regarding the protection of native wild stocks, importation and use of non-indigenous species, aquatic animal health, use of drugs and chemicals, and ownership of the cultured organisms. Additionally, the effects marine aquaculture may have on the aquatic environment must also be addressed.

Much has been published over the last 10 yr on the environmental impacts of marine aquaculture (see, e.g., Ackefors and Sodergren 1985, Weston 1986, Rosenthal et *al.* 1988, DeVoe 1992). However, ecological concerns had been raised by a number of authors a decade earlier (see Odum 1974, Ackefors and Rosen 1979). For instance, three major impacts were identified by Odum (1974): aquaculture as a pollution source, the introduction of exotics and physical alteration of the environment. The latter has not emerged as a critical issue, although these alterations could involve changes in circulation patterns within estuaries, increased sedimentation from poorly designed dredging and filling, interference with freshwater input to the estuary, destruction of productive land peripheral to the estuary, and permanent removal of productive estuarine areas (Odum 1974). One of the challenges to the marine aquaculture industry in the United States will be the success (or failure) of addressing environmental sustainability issues (Chamberlain and Rosenthal 1995).

COASTAL ZONE CONFLICTS

While cultmists, scientists and resource managers face the task of resolving these issues through research studies, monitoring programs and technical assistance support, the marine aquaculture industry continues to deal with its "growing pains." In a recent survey of state aquaculture coordinators, industry representatives and extension specialists, Sandifer (1994) found that only nine out of the country's 24 coastal states and five territories reported moderate growth of marine aquaculture, while 12 reported very slow growth and eight no growth. Asked to identify the major factors responsible for this situation, the respondents indicated that of 12 limiting factors, the top three were use conflicts (92%), permitting (92%) and the regulatory environment (88%) (Sandifer 1994).

Regulations and permitting have often been identified as the principal impediment to the growth of marine aquaculture (McCoy 1989, JLSA 1989, Zieman *et al.* 1990, Hopkins 1991, NRC 1992). But it is the underlying issues that underscore the problem. The NRC (1992) identified eight issues that have contributed to the current situation: (1) difficulties and costs of using coastal and ocean space; (2) public concerns about environmental effects of wastes on water quality; (3) conflicts with other users of the coastal zone; (4) increasing population with concomitant increases in pressures on coastal areas; (5) limited number of sites with suitable water quality; (6) objections from coastal property owners to marine aquaculture installations on aesthetic grounds; (7) broad ecological issues, including concerns about genetic dilution of wild stocks and transfer of diseases through the transport and escape of cultured animals; and (8) limited understanding of the biological criteria needed for the design of viable systems.

Use conflicts represent one of the primary issues marine aquaculturists in the United States must face and are likely to become more pronounced and frequent in the future (Chamberlain and Rosenthal 1995). DeVoe et *al.* *(1992)* found through a survey of the marine aquaculture industry and state regulatory agencies that the competing use of the coastal zone by recreational users, commercial fishermen and developers was frequently encountered. The escalating costs of acquiring access to coastal lands and waters in the country exacerbate the problem. While Japan continues to focus use of its coastal and marine resources on food production, the United States has not made this commitment. As a result, marine aquaculture's place among the many uses of the coastal zone in this country is as yet undefined.

THE LEGAL AND REGULATORY STRUCTURE

The current regulatory environment for marine aquaculture in the United States is a major constraint to its development (NRC 1978, 1992; JSA 1993). No formal federal framework exists to govern the leasing and development of private commercial aquaculture activities in public waters (NRC 1992). For instance, because commercial aquaculture is in the early stages of development, regulators have tended to classify fish farming as an industrial activity requiring water treatment different from other

forms of agriculture (Ewart *et al.* 1995). These factors, along with a general unfamilarity with aquaculture production technologies, waste characteristics and their impact on different categories of receiving waters have precluded the development of uniform standards and policies based on technical data and environmental risk assessment (Ewart *et al.* 1995).

In a 1981 study commissioned by the U.S. JSA of the Federal Coordinating Council on Science Engineering and Technology, the Aspen Corporation examined the federal and state regulatory framework for aquaculture (Aspen Corp. 1981). As many as 11 federal agencies are directly involved in regulating aquaculture and another 10 are indirectly involved. However, only a limited number of permitting and licensing requirements are directly imposed by federal agencies. More characteristic are federal agency programs that indirectly regulate fish farmers (e.g., restrictions on drug use, federal laws administered by states, etc.).

Some 50 federal statutes (with accompanying regulations) were found to have a direct impact on the aquaculture industry, although the actual number of statutes that affect an individual operation will vary depending on its size, site location, the species being cultured and other factors. In total, over 120 statutory programs of the federal government were found to significantly affect aquaculture development. Slightly one-half require a direct compliance response from the fish farmer.

The majority of laws and regulations that specifically authorize, permit or control aquaculture are usually found at the state level. The study examined 32 state regulatory programs and discovered that over 1,200 state laws have some significant bearing on aquaculture operations. Policies and regulations were found to affect aquaculture in eight major areas: aquaculture species use; water quality; water use; land use; facility and hatchery management; processing; financial assistance; and occupational safety and health.

The complexity that results from the involvement of many federal, state and local agencies responsible for all aspects (including advocacy, promotion, conduct and regulation) of marine aquaculture leads to an array of planning acts, policies and regulations (NRC 1992). Federal laws are applied differently in various geographic regions of the country (NRC 1978), and the industry remains concerned about the lack of coordination among agencies regulating aquaculture (JSA 1993). Unfortunately, the federal government has yet to make any significant headway in reducing regulatory constraints (McCoy 1989).

Federal agencies which establish the "ground rules" that most state agencies must follow have adopted vague, confusing and poorly conceived regulations, or none at all (McCoy 1989). This translates into inconsistencies in the development and application of laws and regulations at the state level (deFur and Radar 1995). Few states have a comprehensive regulatory plan which satisfactorily balances economic development and environmental protection. As a result, regulations governing aquaculture are scattered throughout state statutes and do not necessarily fit aquaculture (Breaux 1992). Complicating matters is the fact that existing permit programs do not have provisions for determining the capacity of the coastal and estuarine system for aquaculture, land-based or *in situ* (deFur and Radar 1995).

Major aquaculture problems that arise from state laws and regulations are caused by the lack of uniformity of laws among the states, the shear number of permits, licenses and certifications that must be obtained, and the difficulty in obtaining them (NRC 1978, 1992). Each state has its own unique legal, political and economic climate for aquaculture, and culturists must navigate the regulatory environment differently in each. Only a few states have developed the information management capability to present the applicant with a comprehensive list of all the legal requirements that must be met. State regulatory programs can be and are usually more restrictive than federal guidelines and regulations dictate. The result is that state agencies vary greatly as to what standards they apply to aquaculture (McCoy 1989), and some still apply laws designed for other applications such as those for public fisheries management (NRC 1978, 1992).

Another limitation to the current regulatory regime for marine aquaculture in the United States is the lack of long-range and whole system planning (deFur and Radar 1995). Aquaculture policy appears to be made by granting permits on a case-by-case basis (Rubino and Wilson 1993), and the requirements are often determined using regulations and technical standards not originally developed or intended for aquaculture (Ewart et *al,* 1995). Each permit is considered individually by the issuing agency, usually with no provision for examining cumulative impacts (deFur and Radar 1995).

A final regulatory issue limiting marine aquaculture's growth is the time and cost involved in obtaining permits and licenses. According to McCoy (1989), it may take some four yr or more to obtain the necessary permits for startup. A prospective aquaculture operation could be required to spend over $100,000 in legal and consultant fees to obtain permits (McCoy 1989). For instance, the first applicant for an NPDES (National Pollutant Discharge Elimination System) permit spent $150,000 for environmental assessments and legal fees relating to the processing of his permit (Zieman *et al.* 1990).

SOUTH CAROLINA: A CASE STUDY MARINE AQUACULTURE IN SOUTH CAROLINA

South Carolina is well suited for aquaculture development. Along the coast, the state's 80,000 ha of estuarine

Table 1. Aquaculture production (in kg) and ex-pond value (in $U.S.) of selected species by South Carolina commercial producers from 1989 to 1994.

Pounds of:	1989	1990	1991	1992	1993	1994
Catfish	15,331	101,650	132,857	54,567	136,078	183,433
Crawfish	21,545	18,144	18,144	19,278	13,608	11,340
Hybrid striped bass	N/A	259	5,080	22,680	4,672	11,340
Marine shrimp	12,424	33,371	26,281	38,038	49,895	45,359
Hard clams	*	*	*	*	DNA	DNA
Value of:						
Catfish	236,000	2,569,000	1,788,000	796,000	2,100,000	3,154,000
Crawfish	589,000	400,000	600,000	595,000	645,000	525,000
Hybrid striped bass	N/A	18,810	336,000	1,125,000	252,400	662,000
Marine shrimp	753,600	1,839,300	1,419,500	1,928,800	2,300,000	3,000,000
Hard clams	*	*	*	*	DNA	900,000

DNA = data not available

area, 30,000 ha of wetland impoundments and over 4000 km of tidal creeks are potentially available as production sites (JLSA 1989). Its coastal waters are of high quality; 79% are designated as suitable for shellfish harvesting (Knowles 1988). The mild climate makes the culture of warm-water species feasible.

The true emergence of aquaculture as a viable industry in South Carolina occurred in the early 1980s with commercial production of cultured species of penaeid shrimp (*Penaeus* spp.), catfish (*Ictalurus punctatus*) and crawfish (*Procambarus* spp.). Since that time, hard clam (*Mercenaria mercenaria)* and striped bass (*Morone saxatilus*) hybrid aquaculture have developed The state's Joint Legislative Subcommittee on Aquaculture (JSLA) (1989) identified spotted seatrout (*Cynoscion nebulosus*), redfish (or channel bass, *Sciaenops ocellata*), sturgeon *(Acipenser* spp.). blue crab (*Callinectus sapidus*), bay scallops (*Argopecten irradians)* and the American oyster *(Crassosrrea virginica*) *as* prime marine aquaculture candidates.

Table 1 illustrates annual production (in kg) and ex-pond value (in $U.S.) of selected species produced by South Carolina's private commercial aquaculture industry since 1989. Production is dominated by freshwater species, although marine shrimp has become a major component by value of the industry. Hard clam aquaculture production has been minimal until 1993, the year that Atlantic LittleNeck ClamFarms (ALC) produced its first commercial harvest. The ALC expects to culture and market some 25 to 30 million clams in 1995 (J.J. Manzi, per commun.). According to Rhodes (1993), South Carolina aquaculture could have a $17 million impact, in 1992 dollars, on the state's economy by the year 2000.

South Carolina has demonstrated its commitment to aquaculture development in a number of ways. The S.C. General Assembly stated in 1985 that "aquaculture has the potential for augmenting existing commercial and recreational fisheries and for producing other renewable resources, thereby assisting the state of South Carolina in meeting its food needs and contributing to the reduction of foreign seafood imports into South Carolina and the United States. It is, therefore, in the state's interest, and it is the state's policy, to encourage the development of aquaculture in South Carolina" (Title 2, Chapter 22, Amendments, S.C. Code of Laws).

In addition, a major financial contribution was made by the state to fund the construction and operation of the $4 million James M. Waddell, Jr. Mariculture Research and Development Center. The Center, which includes a 929 m^2 research building, a 242 m^2 maturation building, a 2,323 m2 outdoor tank pad and 25 experimental ponds ranging in size from 0.13 ha to 2.5 ha serves as a major focal point for mariculture research and technology transfer programs in the state and region.

In the 10 yr since the state formalized its position in support of aquaculture, a number of efforts have been undertaken to enhance the growth of the industry, Nevertheless, aquaculture development, particularly in the marine and coastal regions of the state, continues to be limited by the complex regulatory structure, user conflicts and the frequent emergence of unanticipated issues. The regulatory structure and permitting process for marine aquaculture in South Carolina are briefly reviewed below to illustrate these issues.

REGULATION OF MARINE AQUACULTURE IN SOUTH CAROLINA

Marine aquaculture represents a fairly new use of the coastal resources in South Carolina. Its success is predicated on the use of a variety of natural resources. Local, state and federal regulatory agencies seek to allocate these natural resource needs through a permitting system. In theory, by incorporating both agency and public comment in the permitting process, the interests of the aquaculturist, other resource users and the general public can be consider& in decision-making (JLSA 1989).

LOCAL LEVEL

As previously mentioned, many of the regulations that affect aquaculture are found at the state level. This is not to say that local, municipal and federal laws and policies are significant, however. Towns, cities and counties have responsibilities to their citizens to provide orderly development and police power protection. However, most do not formally recognize aquaculture per se and, in many cases, have a difficult time determining where it falls within their master plans and zoning regulations. Indeed, some local governments consider aquaculture an agricultural activity, while others may classify it as a commercial or even industrial enterprise. Even after attempts to educate local governing boards and citizens, aquaculturists may still face major obstacles in some communities due to concerns about water quality, aesthetics and overall quality of life issues.

FEDERAL LEVEL

At the other end of the spectrum, seven federal agencies have regulatory programs that directly affect the marine aquaculture industry: the U.S. Army Corps of Engineers, the U.S. Environmental Protection Agency, the U.S. Fish and Wildlife Service, the U.S. Food and Drug Administration, the U.S. Department of Agriculture, the U.S. National Marine Fisheries Service, and the U.S. Coast Guard (Table 2). Federal oversight of the marine aquaculture industry is fragmented; there is no overall federal framework to address aquaculture development in the coastal zone. Further, while recent evaluations of marine aquaculture suggest that offshore locations may represent a viable alternative (NRC 1992). no formal policies have been developed to manage aquaculture development in the U.S. Exclusive Economic Zone. As a result, existing federal policies vary from one agency to another (and may even differ among divisions within the same agency) and the permitting process car! be time-consuming and quite confusing.

STATE LEVEL

The complexity of the permitting process in South Carolina for marine aquaculture lies in the diversity of agencies involved and their general lack of knowledge of the industry. Twelve agencies and divisions of state government are involved in the regulation of marine aquaculture, concerned with the use of state lands and navigable waters, protection of water quality and quantity, use of aquatic organisms, including exotic species, and other issues (Table 3). Prior to state government restructuring in 1993, the S.C. coastal Council and the S.C. Water Resources Commission were responsible for managing all state lands and waters in the public trust, and regulating the nature and location of water-dependent structures. The S.C. Department of Health and Environmental Control (SCDHEC) implements the provisions of the National Pollutant Discharge Elimination System and the Section 401 Water Quality Certification Program, as established by the U.S. Environmental Protection Agency under the Clean Water Act of 1977 and its amendments, and is also responsible for maintaining shellfish sanitation standards. The SC. Wildlife and Maxine Resources Department (now the S.C. Department of Natural Resources; see below) regulates the use of the state's tidal mud flats and bottoms for the placement of aquaculture structures, and is responsible for all finfish and shellfish permits (for red drum, spotted seatrout, flounder, marine shrimp, hard clams and oysters), boat and equipment permits, and dealer/processor licenses. Other state agencies are involved as well (see Table 3).

Another factor that has added to the complexity of the state's aquaculture regulatory process is the division of agency responsibilities over permitted activities in public waters. The state is divided into three permitting zones: Zone A represents the inland portion of the state excluding the eight coastal counties; Zone B represents areas within the eight coastal counties excluding the "critical area;" and Zone C represents the "critical area" (Fig. 1). The "critical area" is defined by the S.C. Coastal Management Act of 1977 to include all coastal waters, tidelands, beaches and primary oceanfront sand dunes seaward of a boundary line as determined by the state's coastal zone management agency (Section48-39-10 et seq., S.C. Code). Prior to 1994, the S.C. Water Resources Commission had sole responsibility for permitting in Zone A and the S.C. Coastal Council was solely responsible in Zone C. Proposed activities in Zone B required an applicant to obtain a permit from the S.C. Water Resources Commission and a certification from the S.C. Coastal Council that the activities were consistent with the policies of the state's Coastal Zone Management Plan. If certification was denied, the permit could not be issued. Therefore, the location where an aquaculture operation was proposed dictated the regulatory process to be followed and the agencies to be involved.

In addition to the confusion concerning South Carolina's permitting process, the costs in money and time to obtain requisite permits, licenses and certificates has constrained

Table 2. Federal (U.S.) agencies with regulatory programs that impact the marine aquaculture industry (adapted from Breaux 1992).

Agency	Regulatory Responsibility
U.S. Army Corps of Engineers (COE):	*Section 10 Permit - required for any structure and work in or affecting navigable waters (Rivers and Harbors Act of 1899, 33 U.S.C.403)
	*Section 404 Permit - required for the discharge of dredge or fill material into U.S. waters including wetlands (Clean Water Act, 33 U.S.C. 1344, Section 301). Before this permit can be issued, a certification from the responsible state agency is required stating taht the proposed activity would not cause a violation of the state's water quality standards.
U.S. Environmental Protection Agency (EPA):	*National Pollutant Discharge Elimination System (NPDES) - prohibits the discharge of any pollutant from any "point source" into the waters of the U.S. without a permit from the state agency administering the Elimination Act within the state (S.C. Department of Health and Environmental Control, SCDHEC).
	*Use and Application of Pesticides- through the registration and establishment of tolerance levels. Approvals and possibly permits may be required from EPA and SCDHEC.
	*Commenting agency on COE permit applications.
U.S. Fish & Wildlife Service (FWS):	*Fish and Wildlife Import and Export License - required for anyone who imports or exports animals or fish for the purposes of propagation or sale with a value of more than $25,000 a year.
	*Commenting agency on COE permit applications.
U.S. Food & Drug Administration (FDA):	*Drug reglations - affect the use of chemicals as additives to feed as well as chemicals used for the treatment of disease and parasite infections. Separate approval for drug or chemical use for each species cultured is required.
U.S. Department of Agriculture (USDA):	*Vaccine regulations - approval of all vaccines uied in an aquaculture operation must be obtained Very few vaccines are registered for use in this manner, due to the time-consuming and costly process. Again, each vaccine must be separately certified for each species.
U.S. National Marine Fisheries Service (NMFS):	*Fisheries regulations - can affect the potential of marine aquaculture in the nation's exclusive economic zone.
	*Commenting agency on COE permit applications.
U.S. Coast Guard (USCG):	*Protection of Navigation - including the marking of any structure located in navigable waters of the United States.

Table 3. South Carolina natural resources agencies involved in regulating aquaculture development (prior to agency reorganization in 1994).

Function	Agencies
State lands and navigable waters:	1. S.C. coastal Council - "Critical Area" Permit (Zone C) - Coastal Zone Certification (Zone B) 2. S.C. Water Resources Commission - Navigable Water Permit (Zone A) 3. S.C. Land Resources Conservation Commision - Stormwater Management Permit
Protection of water resources:	1. S.C. Dept. of Health & Environmental Control (Water Pollution Control) - Construction Permit - National Pollution Discharge Elimination System Permit - Section 401 Water Quality Certification 2. S.C. Water Resources Commision - Groundwater Use Permit
Use of aquatic organisms:	1. S.C. Wildlife & Marine Resources Dept. (Marine Resources) - Shellfish/Mariculture Permit - Mariculture Finfish Permits - Mariculture Shellfish Permits - Shellfish Harvesting Permit - Harvesting Equipment Permits - Dealer/Processor Licenses 2. S.C. Wildlife & Marine Resources Dept. (Freshwater Fisheries & Wildlife) - Gamefish Breeder's License 3. S.C. Wildlife & Marine Resources Dept. (Executive Office) - Importation of Exotic Species Permit - Hybrid Striped Bass Aquaculture Certificate and Permits 4. S.C. Dept. of Health & Environmental Control (Water Pollution Control) - Shellfish Sanitation Certificates and Permits
Other:	1. S.C. Department of Archives and History - Protection of Archeological Sites and Artifacts 2. State Attorney General's Office - Determination of Clear Title to "Private" Submerged Lands (e.g., coastal wetland impoundments 3. S.C. Dept. of Agriculture - Inspections of Processing Facilities to Ensure Compliance with Good Manufacting Practices

the industry as well. An applicant can spend over $2,800 in one-time application fees and $5,300 in additional fees and rents annually. If water quality monitoring, legal assistance and consultants are necessary, these costs can be 10 to 20 times more expensive (Table 4). Just as consuming is the time it can take for permit applications to be processed and agency decisions to be made.

The NPDES permitting process in South Carolina is a case in point Administered by the SCDHEC, an NPDES permit is required from any "person discharging or proposing to discharge wastes into the waters of the state..." under the state's Pollution Control Act (Act No. 1157, Chapter 1, Title 48, S.C. Code). The normal processing time for an NPDES permit is stated to be approximately two months; however, if a public hearing is necessary or the permit is adjudicated, the processing time could be extended (Fig. 2). Nevertheless, NPDES permitting for aquaculture facilities in South Carolina continues to be a source of contention between the SCDHEC and the aquaculture industry (DeVoe 1994). The S.C. Farm Bureau continues to identify it as one of its top policy concerns [and has each year since 1986 (DeVoe 1994, J. Whetstone, pers.

Table 4. Terms and costs of permits for aquaculture in South Carolina.

Permit	Term	Fee	Notes
Local permits	One time/annual	$200-500	Fees, licenses
Federal permits	One time	$100	Application fee
State permits			
Navigable waters permit	One time	$500	Application fee
Critical area permit (in CZ)	One time	$200	Application fee
Section 401 certification	One time	$500	
NPDES permit (discharge)	Annual	$400-2400	
Stormwater management permit	One time	≤$1000	
Shellfish/mariculture permit	One time	$25	Application fee
	Annual	$5/ac	Up to 500 acres
Hybrid striped bass permit	Annual	$100	First year plus
		$25	annually thereafter
Shellfish harvesting license	Annual	$25.50	
Boat license	Annual	$20	Boats <18 ft
		$25	Boats >18 feet
Equipment license	Annual	$10	Each piece
Wholesale seafood dealer license	Annual	$50	
Land and sell license	Annual	$25	
Other costs:			
Water quality monitoring	Annual	$varies	Required under NPDES
Legal fees	One time	$varies	Hourly fee
Consultants	One time	$varies	Hourly fee

commun.)] due to two primary issues: (1) the time it has actually taken SCDHEC to review and render decisions on NPDES permits for aquaculture has averaged four months or more; and (2) the annual fees for an NPDES permit have increased from a range of $200 to $800 in 1992 (depending on the discharge volume and number of "pipes"), to $400 to $1,600 or higher in 1995.

STATE RESPONSE TO ADDRESS MARINE AQUACULTURE REGULATORY ISSUES

Prior to 1985, when marine aquaculture was beginning to emerge, there were no state policies or programs in place to ease the burdens facing the industry. Since that time, several policy actions have been initiated by the General Assembly and state agencies to address the regulatory complexity, and limiting nature of the administrative and bureaucratic structure of the state. As noted above, the S.C. General Assembly did pass legislation in 1985 which for the first time acknowledged the existence and potential of the fledging aquaculture industry (Chapter 22, Title 2, S.C. Code). A Joint Legislative Committee on Aquaculture was formed to foster needed legislation through the S.C. General Assembly and an Interagency Advisory Staff Group was established to offer advice and information to the Joint Committee. Also called for was the development of a statewide aquaculture plan. Thus the process by which the state began to respond to the needs of the aquaculture industry was underway; the critical analysis presented below of these actions and their impact on the industry suggests to this author that the state of South Carolina, while being acknowledged as having made "significant progress in streamlining the regulatory and permitting constraints affecting aquaculture" (Breaux 1992), still has much to accomplish.

DEVELOPMENT OF AN AQUACULTURE PERMITTING GUIDEBOOK

The regulatory and permitting maze that faced prospective aquaculturists in South Carolina during the early 1980s was overbearing for three reasons. First, no regulatory framework existed for the aquaculture industry. Second, the permitting agencies did not understand the industry or the regulatory requirements necessary to balance the needs of the industry with other users. Third, there was no single, consolidated source of information available on the regulatory process. As a result, the industry had no guidance in

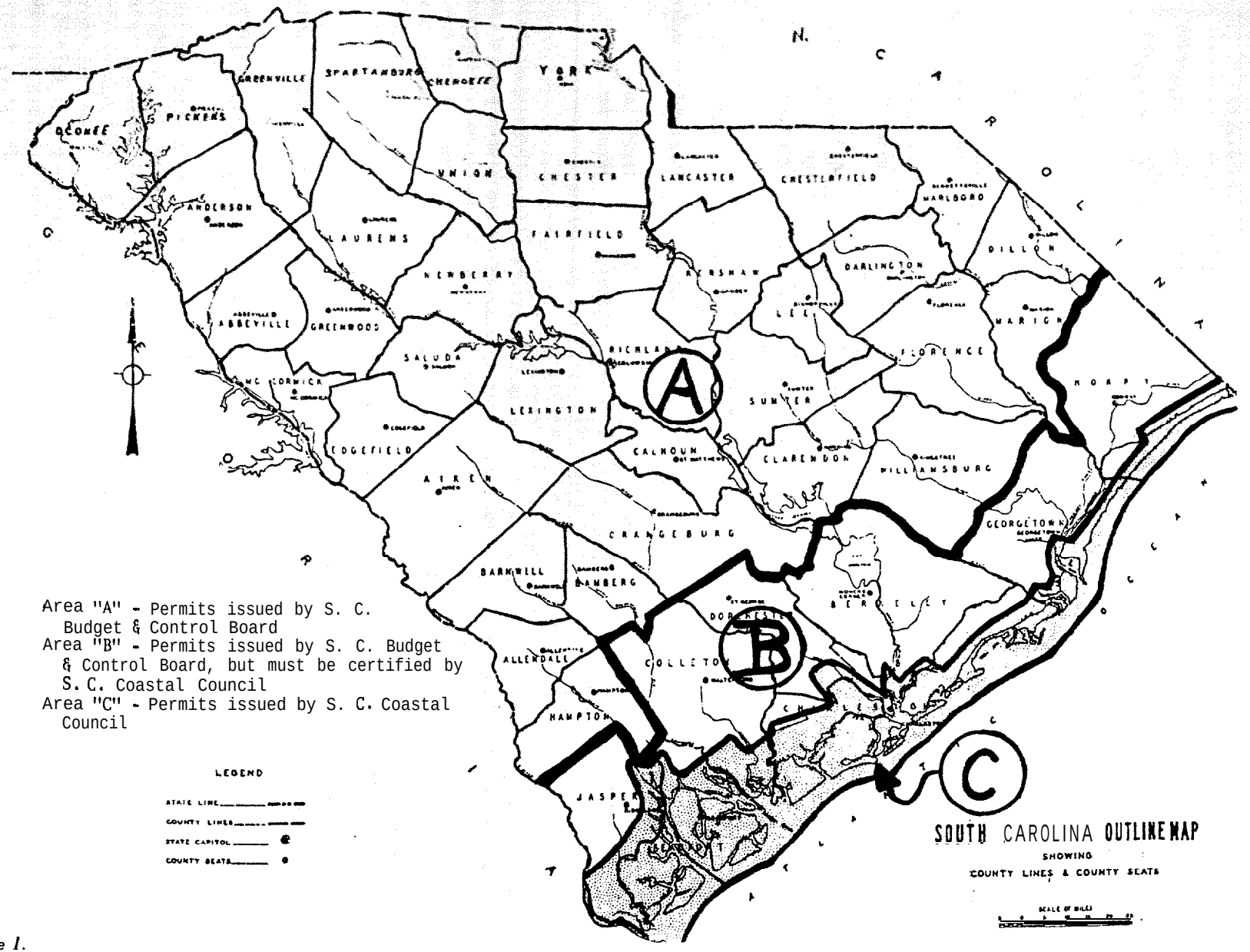
N. C A R O L I N A
OCONEE
PICKENS
GREENVILLE
SPARTANBURG
CHEROKEE
YORK
UNION
CHESTER
LANCASTER
CHESTERFIELD
MARLBORO
ANDERSON
LAURENS
FAIRFIELD
NEWBERRY
DARLINGTON
DILLON
ABBEVILLE
GREENWOOD
SALUDA
LEE
FLORENCE
MARION
MC CORMICK
LEXINGTON
SUMTER
EDGEFIELD
CALHOUN
CLARENDON
WILLIAMSBURG
AIKEN
ORANGEBURG
GEORGETOWN
BARNWELL
BAMBERG
BERKELEY
ALLENDALE
COLLETON
HAMPTON
JASPER
A
B
C
Area "A" - Permits issued by S. C. Budget & Control Board
Area "B" - Permits issued by S. C. Budget & Control Board, but must be certified by S. C. Coastal Council
Area "C" - Permits issued by S. C. Coastal Council
LEGEND
STATE LINE
COUNTY LINES
STATE CAPITOL
COUNTY SEATS
SOUTH CAROLINA OUTLINE MAP
SHOWING
COUNTY LINES & COUNTY SEATS
SCALE OF MILES

Figure 1.

navigating the regulatory seas, and the agencies had no basis upon which to guide the industry through the process.

In 1983, an ad hoc Committee on Aquaculture Permitting, consisting of state agency officials, extension specialists and industry representatives, was convened to address these issues. It took the committee almost two yr to unravel the permitting process for aquaculture and, in late 1984, an *Interim Guide to Aquaculture Permitting in South Carolina* was published (DeVoe and Whetstone 1984). The purpose of the guide, which has been updated twice, is to provide prospective applicants assistance in meeting regulatory requirements. More than 300 copies of the guide have been distributed upon request and has served as a model for guidebooks developed by several other states.

However, there are several limitations to the utility of such a publication. In South Carolina's case, the guide was published for use by the aquaculture community only until a formal state permitting mechanism was established; this has yet to occur. Another problem relates to the regulatory and permitting environment itself-it is in a dynamic state of evolution. Laws and regulations continue to be passed and amended. As a result, the guide is in continuous need of revision. This can be a costly process, and a decision as to who pays must also be made.

DESIGNATION OF AQUACULTURE AS AGRICULTURE

The S.C. General Assembly passed legislation in 1986 which declared that the terms "agriculture, agricultural purposes, agricultural uses, farm crops, cultivated crops or words of similar impact shall include...aquaculture" (Sec. 46-140, 1976 Code). The aquaculture industry in South Carolina, as well as throughout the United States, strongly supports this designation as it gives state departments of agriculture more of a role over private aquacultural activities. Since 1986, aquaculturists have benefitted from a number of USDA and state agriculture department programs, including numerous sales tax exemptions, access to farm loan programs and additional technical assistance provided through the Agricultural Stabilization and Conservation Service and other USDA agencies.

However, it appears that many in the aquaculture industry are looking to the federal and state departments of agriculture as "shields" from excessive environmental regulation. As long as aquacultural practices involve the use of public resources (tidelands, waters, wetlands, etc.), the industry will most likely continue to be subject to the laws and regulations of federal and state agencies that seek to protect these public resources.

CREATION OF AN AQUACULTURE PERMIT ASSISTANCE OFFICE

The S.C. General Assembly emphasized the importance of providing permitting assistance to the aquaculture industry through the creation, in 1988, of the Aquaculture Permit Assistance Office (Title 46, Chapter 51, S.C. Code). This legislation established the position of Permit Facilitator within the S.C. Department of Agriculture to assist culturists in: (1) obtaining permits; (2) obtaining technical assistance from state, private, and academic institutions; (3) understanding new changes in state or federal regulations that may affect the outcome of a permit application; and (4) obtaining application forms.

In addition, the legislation required that the executive directors of all state agencies involved in regulating aquaculture convene to develop a single application form which "must be used by all the permitting agencies" (Title 46, Chapter 51, S.C. Code). It requires the agencies to refer any individual seeking permits for aquaculture to the Aquaculture Permit Assistance Office to complete an application and provide all information required by the permitting agencies to process the application and render a decision.

The creation of the Aquaculture Permit Assistance Office has greatly enhanced the ability of small-scale culturists to traverse the regulatory process. The permit facilitator has essentially eliminated the time it had taken for culturists to identify the process to be followed and the agencies with jurisdiction. While the application process has been streamlined. the fact remains that a myriad of permits, certifications and licenses are still required. Improving the "front end" of the process has not affected the time, cost and complexity of the regulatory structure for aquaculture. In addition, the permit facilitator has no direct permitting authority, so while (s)he can assist the culturist administratively, (s)he cannot affect permitting decisions. Finally, the time savings that accrue to prospective culturists are solely front-end; once the application is received by the permit facilitator and sent for processing. it follows the normal permitting timetable regularly used by the agencies.

PLANNING FOR AQUACULTURE DEVELOPMENT

The S.C. General Assembly, in creating the Joint Legislative Committee on Aquaculture, required its staff to prepare and periodically update a state aquaculture development plan to include an assessment of resources, opportunities and constraints to foster interagency and institutional cooperation in the development of aquaculture (Title 2, Chapter 22. S.C. Code). *The Strategic Plan for Aquaculture Development in South Carolina* (1989) was prepared to: (1) identify existing private and public sector aquaculture activities in South Carolina; (2) outline a development program for commercial aquaculture and its required public sector assistance; and (3) identify factors limiting aquaculture development in South Carolina and

Table 5. South Carolina state government reorganization of the natural resources agencies.

Major department	Offices and departments
1. S.C. Dept. of Natural Resources	a. S.C. Wildlife & Marine Resources Dept. b. S.C. Land Resources Conservation Commission (NR) c. S.C. Water Resources Commission (NR) d. Migratory Waterfowl Commission e. Geological Mapping (from Budget & Control Board) f. State Geologist g. Natural Resources Police (law enforcement)
2. S.C. Dept. of Health & Environmental Control	a. S.C. Dept. of Health & Environmental Control b. S.C. Land Resources Conservation Commission (R) c. S.C. Water Resources Commission (R) d. S.C. Coastal Council (coastal zone management) e. S.C. Mining Council

NR = non-regulatory; R = regulatory

formulate a plan to remove constraints and stimulate commercial development.

The strategic plan identified five major factors that require attention (regulatory constraints, environmental concerns, financial needs, marketing restrictions, and knowledge and information) and offered 41 recommendations to enhance aquaculture's development. It represented the first major comprehensive evaluation of the aquaculture industry performed in the state. The plan also identified specific action steps needed to address the recommendations and assigned responsibilities for carrying them out.

As with any plan, implementation is the key. While specific action items and strategies for implementation were included in the plan, no incentives (or disincentives) were offered to ensure the recommendations were addressed. The Joint Legislative Committee on Aquaculture and its Interagency Advisory Staff rarely called for up dates on the status of implementation, and the joint committee itself was dissolved as part of state government reorganization in 1993. So while the aquaculture industry continues to call for plan implementation, it is having more difficulty effecting necessary changes in law and regulations. As a result, only 17 of the 41 recommendations have been addressed in the six yr since the plan was adopted by the S.C. General Assembly.

SOUTH CAROLINA STATE GOVERNMENT REORGANIZATION

Throughout its history, South Carolina has had a strong legislative form of government. Each of the state's 127 agencies reported directly to their respective standing committees in the Senate and House of Representatives, which controlled their programmatic activities and budgets, while the role of the Governor's office had essentially been advisory in nature. By the early 1990s, however, this system was challenged as one that represented "run-away" government, with no centralized authority being held accountable for agency actions and spending.

In 1993, the S.C. General Assembly passed sweeping state government restructuring and reform legislation. Seventy-five of the state's 127 agencies were consolidated into 17, 12 of which were made part of the newly created Governor's Cabinet. The nine natural resources-related agencies in existence prior to 1993 were combined into two: The S.C. Department of Natural Resources and the SCDHEC (Table 5).

One intention of the General Assembly was to ease the burden of the regulatory process on permit applicants by reducing the number of agencies with which they had to deal. Further, the state was seeking to achieve "one-stop" permitting; state government reorganization has provided a regulatory setting where this goal may soon be realized. But as the move toward centralization continues, several issues remain. While "one-stop" permitting may be desirable, it essentially internalizes the permitting process. All permits required prior to government reorganization must still be obtained by the culturist; however, agency decisions on each are now made internally. Opportunities to negotiate permit terms and stipulations are minimized as a result.

Another issue is emerging as well. While the SCDHEC is now the state's primary natural resource regulatory agency, the S.C. Department of Natural Resources has retained all regulatory responsibilities over the state's wildlife and fisheries and, in the case of aquaculture, the state's public tidelands and coastal waters. There appears to be

Possible NPDES and No Discharge System Permit Processing Paths with Estimated Time Frames Shown

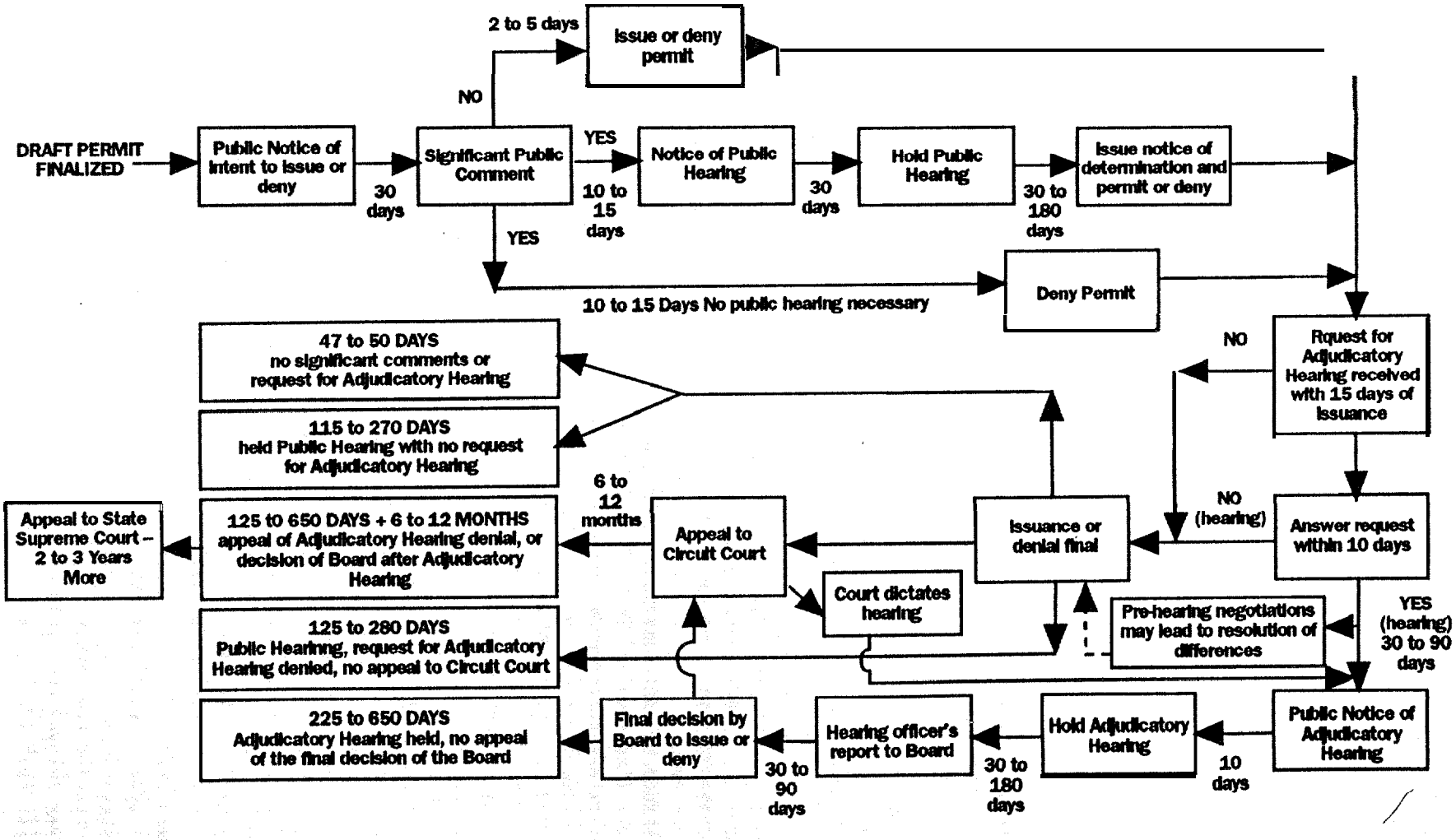

Figure 2

an emerging friction between the two agencies over their respective roles regarding aquaculture development in the state. The legislated responsibilities of the S.C. Department of Agriculture's Aquaculture Permit Assistance Office have come into question as well.

CHANGES IN STATE LEGISLATION AND REGULATION

Much of the marine aquaculture activity occurring in South Carolina would not now exist if the S.C. General Assembly had not passed new or amended existing laws. Overall, the state has responded when necessary to facilitate the development of marine aquaculture operations. For instance, the SC. General Assembly: (1) provided exemptions from seasonal and minimum size regulations to the hard clam aquaculture industry (1986 and 1989); (2) legalized the culture of hybrid striped baas in 1988, after very difficult negotiations that took place over a four Yr period; (3) declared that all fish, shellfish, crustaceans and plants grown in bonafide aquaculture operations remain the private property of the culturist until sold or traded (1989); (4) provided for significant penalties (including fines and imprisonment) for anyone convicted of causing damage to aquaculture facilities or stealing cultured fish and shellfish (1989); (5) developed an importation policy for the use of non-native penaeid shrimp species in culture operations (1990); and (6) is considering coastal zone regulations that allow for the use of the state's waters and tidal bottoms for aquaculture near population centers (proposed for 1996).

In addition, the state's aquaculture industry and agencies are working to develop a General Permit for qualified aquaculture operations under the National Pollutant Discharge Elimination System program and, together with the universities, are beginning to develop the criteria necessary to prepare best management practices (BMPs) for certain forms of marine aquaculture.

South Carolina has obviously demonstrated a willingness to deal with constraints to aquaculture development through legislative and regulatory reform, but it has done so in a reactive, crisis-management mode. This becomes extremely clear when examining the State Code Of Laws—statutes directly affecting aquaculture are spread throughout the Code Book. As a result, there is no overall state frameWork for aquaculture in South Carolina.

THE FUTURE FOR MARINE AQUACULTURE IN THE UNITED STATES

South Carolina is viewed by many as having made significant strides in minimizing regulatory and institutional constraints to marine aquaculture. Nevertheless, the situation in South Carolina is representive of the complexity of the issues that face many coastal states throughout the United States. Progress is occurring throughout the country, albeit at a fairly slow pace. The potential of marine aquaculture remains high as research information and technologies continue to be generated for cultivating a diversity of marine species, ameliorating the environmental effects of the industry and developing cost-effective sustainable culture techniques. Realization of that potential is being severely limited by the institutional and legal constraints presented above.

These issues are not new to the industry. Note the key conclusions of the NRC panels that, in 1978 and 1992, met to review the potential and growth of the U.S. aquaculture industry. In 1978, an NRC panel concluded that constraints on the development of the U.S. aquaculture industry "tend to be political and administrative, rather than scientific and technological" (NRC 1978). Fourteen years later, the NRC stated that "solutions to the environmental problems constraining marine aquaculture will involve approaches that combine technological 'fixes' with improved regulatory and management structures, as well as public education..." (NRC 1992). It is certainly unfortunate that while these issues were fully explored in the late 1970s, many still remained in 1992 and do so today.

A number of proposals have been offered over the last four yr to remove these constraints and move the industry foward. The NRC (1992) suggested that, among other things, the U.S. Congress should: (1) designate marine aquaculture as a recognized use of the coastal zone; (2) create a legal framework to address constraints; (3) modify federal regulations that now limit development of marine aquaculture; (4) create a congressional committee on aquaculture; and (5) explore opportunities offered by onshore and offshore aquaculture.

At the state level, Rubino and Wilson (1993) recommended that: (1) aquaculture be defined as agriculture in law; (2) a lead agency be identified in each state to coordinate regulatory programs; (3) the permitting process be streamlined; (4) conflict resolution measures be adopted; (5) aquaculture be included in government planning; (6) regulations be formulated in consultation with the industry; (7) adoption of best management plans be encouraged; and (8) research, education and extension efforts be supported and expanded.

The key, however, to the future of marine aquaculture in the United States is the creation of technological and political systems that will provide for sustainable marine aquaculture. Sustainable aquaculture will only be achieved if all facets of the industry-production and technology, economics and marketing, business and financing, natural resource needs and protections, and administrative and legal institutions—are dea& with simultaneously. This is a lofty goal, as the difficulty lies in the details of how exactly to attain it, as those details differ with different modes of aquaculture (Bardach 1995). Nevertheless, education and communication will be the primary tools required to

move toward a viable and sustainable marine aquaculture industry in the United States.

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Effects of Fish Farming on Macroinvertebrates: Comparison of Three Localities Suffering from Hypoxia

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ABSTRACT

To clarify the effects of fish farming on macrofauna, quantitative samples of the macrofauna and sediments were obtained from Gokasho Bay in the spring and summer, and were compared with samples from Osaka Bay and Omura Bay. These three localities suffer from environmental hypoxia as a result of fish farming (Gokasho Bay), sewage and industrial effluent (Osaka Bay), and enclosed topography (Omura Bay). In these localities, density and biomass increases correspond to oxygen recovery in autumn. In Gokasho and Osaka bays, near-azoic conditions were observed in the summer when anaerobic conditions prevailed, but in the winter and spring high population densities (14,700-16,000 ind/m^{-2}) were encountered. These high densities are primarily due to the occurrence of an overwhelmingly dominant species, i.e., the spionid polychaete ***Pseudopolydora paucibranchiata*** in Gokasho Bay (62.7% of the total number) and the capitellid polychaete ***Capitella*** sp. in Osaka Bay (73.7%). The large populations in these localities may be the result of the rich food source derived from gross organic enrichment, but in Omura Bay low organic input seems to restrain the abundance (1,920 ind/m^{-2}) even when oxygen levels recover. The dominance of ***P. pnucibranchiata,*** which is a suspension- and selective-surface &posit feeder in Gokasho Bay, suggests a large amount of food at the water-sediment interface. This food material originates from the leftovers or feces of cultured fish. However, the dominance of the nonselective subsurface deposit feeder, ***Capitella*** sp., in Osaka Bay may reflect the accumulation of organic debris within the substratum. Thus, the two distinct dominant species indicate the different state of the bottom environments in Gokasho Bay and in Osaka Bay.

INTRODUCTION

As fish farming has developed since the beginning of the 1960s in Japanese coastal waters, there has been a steady rise in levels of water deoxygenation and the occurrence of noxious red tides, which often have caused mortalities of maricultured organisms. It is estimated that 80% of the feed discharges outside of the culture cages, in the form of leftovers (20%) and excretions of fish, i.e., feces (10%) and urine (50%) (Itoh 1994). These organic wastes induce qualitative and quantitative changes in the surrounding macrofauna (Brown et al. 1987, Ritz et al. 1989, Tsutsumi et al. 1991).

In Gokasho Bay, which has aria style coastline with an area of 22.2 km* and a mean depth of 12.7 m (Fig. 1), fish farming has been carried out since the introduction of yellowtail culture in 1962. Thereafter, the culture of red sea bream was incorporated, and since then fish production has progressively increased. In this bay, fish farms are concentrated in a small inlet, where fish cages cover an area of 2.4 ha. In 1993 in this area, 7,800 mt of feed were used for culture and 1,360 mt of fish were produced. This active farming has also unfortunately caused serious problems, such as oxygen deficiencies in the water (Abo and Toda, in press) and blooms of noxious dinoflagellates (Honjo et al. 1991, Toda et al. 1994).

In order to clarify the effects of fish farming on the bottom environment, this study has examined the macrofauna from three localities with hypoxic waters: Gokasho Bay, where there is an abundance of fish farms; Osaka Bay, characterized by heavy sewage pollution; and Omura Bay, where the topography is that of an enclosed bay with restricted water exchange.

MATERIALS AND METHODS

A survey of the macrobenthos was conducted in Gokasho Bay (34•19'N, 136•40'E), the innermost part of Osaka Bay (34•39'N, 136o27'E), and Omura Bay (32•58'N, 129•53'E) (Fig. 1). In each locality, samples were collected on two occasions, i.e., in the deoxygenated season (August-September) and in the oxygen-recovery season (February-April) (Table 1). Sampling procedures were similar throughout the investigations. Two replicate samples were taken at each station with a 0.04-m^2 Ekman-Birge grab and a 1 -mm mesh sieve. The collected animals were identified, the number of individuals of each species counted, and their wet weights determined after blotting

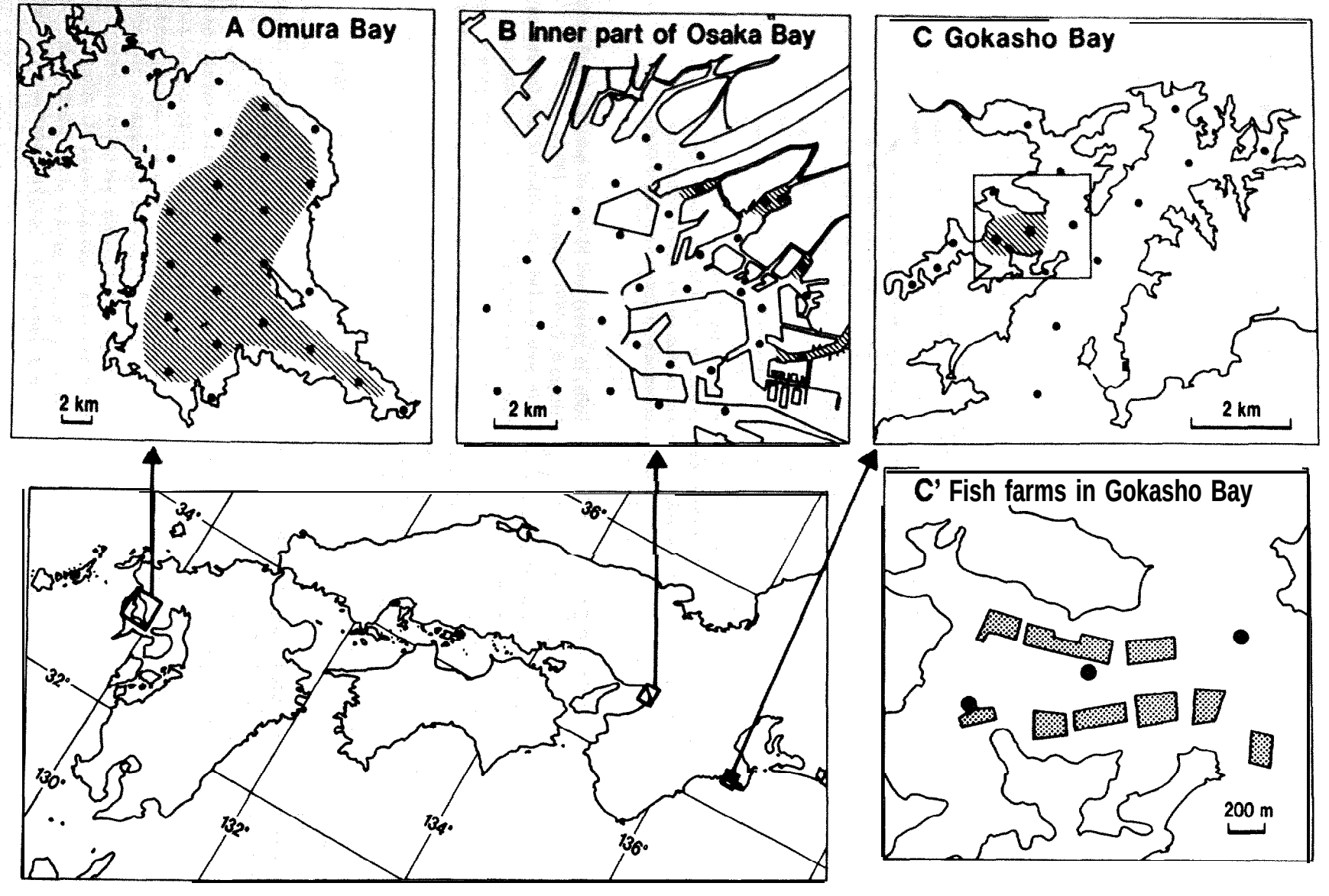

Fig. I. Omura Bay (A), the inner part of Osaka Bay (B), and Gokasho Bay (C,C'), showing sampling stations (filled circles), areas suffering from hypoxia (hatched area), and fish farms in an inlet of Gokasho Bay (C', stippled area).

Table 1. Surveys of the macrobenthos in three localities in Japan.

	Gokasho Bay	Osaka Bay	Omura Bay
Sampling date	20-2 1 Apr 1993	27-29 Aug 1984	20 Aug - 10 Sep 1992
	11-12 Aug 1993	25-27 Feb 1985	22 mar - 1 Apr 1993
No. of stations a	2	4	16
Reference	Yokoyama *et al.* 1996	Yokoyama 1986	Yokoyama 1995a, b
		Yokoyama 1994	
		Yokoyama *et al.* 1985	

"The number of stations under the influence of hypoxic waters.

Table 2. Community parameters of the macrobenthos in three localities in Japan.

		Gokasho Bay	Osaka Bay	Omura Bay
Density (ind/m^{-2})	Feb/Apr	14700	16000	1920
	Aug/Sep	69	3	439
Biomass (gm/m^{-2})	Feb/Apr	57.7	90.5	8.9
	Aug/Sep	0.1	<0.01	2.3
Diversity indicies				
H'(bit)	Feb/Apr	2.5	1.4	3.1
	Aug/Sep	0.7	-a	2.1
H'max	Feb/Apr	5.7	3.9	4.3
	Aug/ Sep	0.8	-a	2.6
J'	Feb/Apr	0.45	0.36	0.72
	Aug/Sep	-a	-a	0.78

[a]Incomputable

with filter paper. Along with the biological sampling, sediment samples were obtained for analysis of particle size, ignition loss (IL,), chemical oxygen demand (COD), and total sulfide content. Dissolved oxygen (DO) of the bottom water (OS- 1.0 m above the bed) was measured using a DO meter (YSI model 58) and the Winkler method.

To present the species diversity, the Shannon-Weaver function H', the species richness index H'max, and the evenness index J' (Pielou 1969) were adopted. Species diversity has components of species richness and evenness; this relation is expressed as H'= H'max X J'.

CHARACTERISTICS OF MACROFAUNA IN THE THREE LOCALITIES

Gokasho Bay is divided into five ecological areas on the basis of species composition. Among them, the inlet area around the fish farms (see Fig. lc') has distinct features in its species composition, community structure, and seasonal fluctuations (Yokoyama 1996). The characteristics of the community parameters (Table 2) and the dominant species (Table 3) in this area are compared with those from the other two localities suffering from environmental hypoxia.

The area around the fish farms in Gokasho Bay is characterized by large concentrations of the spionid polychaete *Pseudopolydom paucibranchiara* which comprised 62.7% of the total number of individuals in April 1993. This species also ranked first in biomass, but the ratio of occupancy was relatively low (21.1%) because of its small size. The dense population of *P. paucibranchiata* and the occurrence of many other species (a total of 67 species), including the polychaetes *Lumbrineris longifolia* and *Prionospio pulchra, the* amphipod *Protomima imitatrix*, and the bivalve Theora fragilis, enhanced the density (14,700 ind/m^{-2}) and the species richness (H'max, 5.7), showing the highest level in the three localities. In August 1993, however, the fauna was limited to a few individuals

Table 3. Dominant and subdominant species (three species ranked highest in density or in biomass) in three localities in Japan during the oxygen-recovery period.

	Species	Ind/m^{-2}	Percent[a]	Species	gm/m^{-2}	Percent[a]
Gokasho Bay	*Pseudopolydora paucibranchiata*	*0210*	*62.7*	*Pseudopolydora paucibranchiata*	*12.2*	*21.1*
	Lumbrineris longifolia	*950*	*6.5*	*Paraprionospio sp.*	*8.5*	*14.7*
	Prionospio pulchra	*619*	*4.2*	*Theora fragilis*	*7.4*	*12.7*
Osaka Bay	*Capitella* sp.	11800	73.7	*Capitella* sp.	73.4	81.1
	Brionospio pulchra	1270	8.0	*Neanthes succinea*	*5.2*	*5.7*
	Nebalia bipes	1110	7.0	*Polydora sp.*	*4.3*	*4.7*
Omura Bay	*Prionospio pulchra*	*733*	*38.2*	*Nectoneanthes latipoda*	*2.3*	*25.8*
	Sigambra sp.	1'55	8.1	*Theora fragilia*	*1.6*	*19.0*
	Ophidromus sp.	145	7.6	*Paraprionospio* sp.	1.1	12.4

[a]Percentage in the total density and in the total biomass of the benthos.

Table 4. Environmental factors in three localities in Japan.

		Gokaso Bay		Osaka Bay		Omura Bay	
		Range	Mean	Range	Mean	Range	Mean
Bottom water							
Dissoved oxygen	Feb/Apr	4.6-S. 1	4.98	3.3-6.1	*4.8*	*8.1-9.4*	*9.0*
(mg/L^{-1})	Aug/Sep	0.8-1.7	1.3	10.4-0.7	0.6	o-4.7	*2.4*
Sediment							
Median diameter (0)		2.6-7.1	4.9	*5.1-6.2*	*5.4*	*5.9-8.3*	*7.5*
Silt-clay fraction (%)		23.3-87.6	158.3	66-70	*73*	70.6-99.1	*93.7*
Ignition loss (%)		8.3-18.2	13.3	12.1-16.3	14.2	9.9-15.5	13.2
COD (mg/gm^{-1} dry)		5.0-31.9	16.3	25.1-38.6	33.1	11.0-33.0	18.8
Total sulfide (mg/gm^{-1} dry)		0.15-0.96	0.58	2.8-12.2	7.1	0.04-0.33	0.17

of only four species (the polychaetes L. longifolia, P. pulchra, Paraprionospio sp., and Monticellina sp.), and the density had decreased drastically to 69 ind/m^{-2}.

In the innermost part of Osaka Bay (delta mouth of the Yodo River), where a large amount of sewage and industrial effluent flows into the water from the densely populated Kyoto-Osaka area, near-azoic conditions prevail from early summer through autumn (Yokoyama et al. 1985, Yokoyama 1994). However, intense recruitment of the capitellid poiychaete *Capitella* sp. during a short period in the winter causes high values in density and in biomass. In February 1985, *Capitella* sp. dominated the density (73.7% of the total number) and the biomass (8 1.1%) (Yokoyama 1986). Other species characteristic for this area include the polychaete I? *pulchra* and the crustacean *Nebalia bipes,* but their ratios of occupancy in the total density or biomass are low, usually less than 8% (Table 3).

Omura Bay, which is a vast, enclosed basin characterized by stagnant water and fine sediments, lacks an overwhelming species dominance, as is the case in the other two localities (Yokoyama 1995a, b). *P. pzdchra* ranked first throughout the sampling period, but it had relatively low abundance percentages, i.e., 38.2% in spring and 30.9% in summer, This is the reason why there are high evenness values (J', 0.72-0.78; Table 2). In Omura Bay, azoic conditions are rarely found in summer, although the values in density and in biomass during the oxygen-recovery season were low (1,920 ind/m^{-2}, 8.9 gm/m^{-2}) compared to the other two localities.

ENVIRONMENTAL FACTORS AFFECTING MACROFAUNA

Physical and chemical factors in the three localities are summarized in Table 4. A characteristic common to these localities is the deoxygenation of the bottom water in the

summer period. However, it is impossible to determine the differences in the degree of deoxygenation between these localities from the present data. Anoxic conditions have frequently been observed in Gokasho Bay (Abo and Toda, in press), Osaka Bay (Tsuruho et al. 1980), and Omura Bay (Akagi and Hirayama 1991). Such deoxygenation must be a dominant factor in the declining density and biomass levels during the summer.

High values of COD (33.1 mg/g^{-1}) and total sulfide content (7.1 mg/g^{-1}) in the sediment in Osaka Bay indicate the extreme deterioration of the bottom environment. In Gokasho Bay, in spite of a large amount of organic wastes from the fish farms, values of IL loss and COD were not as high as in Osaka Bay. This is probably because of the moderate water movements, which are indicated by the relatively small value of the silt-clay fraction (58.3%). The enclosed topography of Omura Bay induces the deposition of fine particles in the main basin (median diameter, 7.5; silt-clay fraction, 93.7%). Such stagnant conditions cause oxygen deficiency in the overlying water, although eutrophication is not as advanced as it is in the other two localities.

In Omura Bay, oxygen was at saturation levels during the spring. In Gokasho and Osaka bays, however, low oxygen conditions remained after the summer, as shown by the low values of oxygen saturation: 6 1% in Gokasho Bay during the spring, 50.5% in Osaka Bay during the winter. The protracted occurrence of oxygen deficiency in Gokasho and Osaka bays suggests the continuous input of large quantities of organic matter.

Organic enrichment in Gokasho and Osaka bays may be favorable in providing a rich food source for macroinvertebrates, but it also deoxygenates the water and subsequently eliminates the benthos in the summer. Macroinvertebrates which have a physiological ability to withstand low oxygen tensions and high sulfide concentrations increase their populations rapidly by utilizing the accumulated organic resources even when oxygen levels do not recover sufficiently. Under these conditions, predators and competitors will be excluded by their low resistance to oxygen deficiency. ***P. paucibranchiata*** and ***Capitella*** sp. are good examples of organisms which can exploit nutrient sources in a harsh environment. Tamaki (1985) reported an extreme increase of ***a P. paucibranchiata*** population within a caged plot, established experimentally on a tidal flat. The environmental conditions around the fish farms in Gokasho Bay may resemble those of the caged plot in preventing predators and/or competitive organisms.

Finer particles of sediment contain a larger amount Of organic matter. However, in general, this organic matter is accumulated over a long period and is highly decomposed, and it probably serves a limited role as a nutrient for macroinvertebrates. Although fine, enriched sediments are deposited on the bottom of Omura Bay, where large values of IL and COD were observed (Table 4), the poor food source seems to restrain the biomass even when oxygen levels recover.

COMPARISON OF THE DOMINANT SPECIES

Distinct dominant species exist in two localities, i.e., ***P. paucibranchiata*** in Gokasho Bay and ***Capitella*** sp. in Osaka Bay (Table 3), although the two localities have similar trends in their community structure and seasonal fluctuations. It is widely held that many pollution indicators have opportunistic life history characteristics such as small size, short generation times, large reproductive rates, and high mortality levels (Grassle and Grassle 1974, McCall 1977, Pearson and Rosenberg 1978). Such characteristics allow these species to quickly colonize denuded areas at any time of the year when the habitat is improving.

Table 5 summarizes the life history traits of the two dominant species. The ***Capitella*** species complex, which is known worldwide as an indicator of heavy pollution, is regarded as a typical opportunist because of its prolonged breeding season, high growth rate, brief maturation period, and high mortality levels after periods of high reproduction (Grassle and Grassle 1976). As for the Japanese species of ***Capitella,*** a similar pattern of life history has been reported (Tsutsumi and Kikuchi 1984, Tsutsumi 1987, Kikuchi 1991). A timetable for the development of ***P. paucibranchiata,*** which was described by Myohara (1980). indicates that this species is also an opportunist; it has a small adult size (5-10 mm in length) and reproduction begins about one month after oviposition. Blake and Woodwick (1975) suggested that ***P. paucibranchiata*** in California reproduces throughout the year, It is, therefore, possible that these two species are dominant in the unstable environment by virtue of their ability to reproduce rapidly at any time of the year.

The two dominant species exhibit dissimilar traits in development (Table 5). A mature female of ***P. paucibranchiata*** reproduces every week (iteroparous), and produces small eggs; larvae hatch from the egg capsule 3-4 days after oviposition at the 3-segment stage, then larvae spend a relatively long planktotrophic pelagic period (2-4 wk). ***Capitella*** sp. in Japan reproduces once during their lifetime (semelparous), and produces a smaller number of larger eggs and lecithotrophic (not feeding) larvae which have a brief pelagic period (<24 h) (Table 5). Such differences in development were discussed by Levin (1984). She suggested that ***P. paucibranchiata*** has an advantage over ***Capitella spp. in*** California in colonizing new habitats by enhanced dispersal mechanisms. She also suggested that Capitella spp. has an advantage in its ability to reduce its mortality levels during the short pelagic period and in its ability to exploit nutrient-rich habitats during

Table 5. Life history **traits** of two dominant **species** in Japan.

	Pseudopolydora paucibranchiata	*Capitella sp.*
Adult size	5-10 mm	15-20 mm
Time to first reproduction	1 month	l-2 months
Frequency of reproduction	Iteroparous	Semelparous
Oocyte size	95-100 m	280-300 m
Brood size	250-500	50-500
Brood protection	Capsule in the tube	Inside the tube wall
Spawning period	Most of the year	Most of the year
Pelagic period	2-4 wk	<24 h
Trophic mode of larva	Lecithotropic (<3-segment stage) Planktotrophic (>3-segment stage)	Lecithotrophic
Feeding mode of adult	Suspension feeding and surface deposit feeding	Subsurface deposit feeding

Data based on Blake and Woodwick (1975), Weinberg (1979), Myohara (1980), Levin (1981, 1984), and Tamaki (1985 for *P. paucibranchiata*; and Tsutsumi and Kikuchi (2984). Tsutsumi (1987), Tsutsumi *et al.* (1990), and Kikuchi (199 1) for *Capitella* sp.

the adult stage of the life cycle. In Gokasho Bay, the azoic area created by oxygen deficiency is readily repopulated by *P. paucibranchiata* through dense settlement of available larvae, which hatch from the surrounding habitats.

Another difference between the two species is their distinctive feeding mode (Table 5). *P. paucibranchiata* makes a tube which projects into the water, and feeds selectively on organic mineral aggregates and suspended organic particles at the sediment-water interface by using a pair of palps (Weinberg 1979, Levin 1981, Tamaki 1985). *Capitella* spp. burrow into the substratum, and feed nonselectively on subsurface sediments containing decayed organic particles and associated microbes (Tenore 1975, Fauchaldand Jumars 1979, Tsutsumi et al. 1990). Such a difference in feeding mode seems to indicate the different state of the bottom environments between Gokasho Bay and Osaka Bay. Active fish farming has been carried out in an inlet of Gokasho Bay, where the input of fish feed is as high as 325 kg/m-2/yr-1 over an area of 2.4 ha. Itoh (1994) estimated that 80% of the food input is loaded into the environment and 30% discharges directly as organic Particles in the form of leftovers and fish feces. It seems likely that suspended organic wastes are sent to the bottom layer and that these particles drift on or above the seabed depending on the degree of water movement. The dense population of *P. paucibranchiata* must be sustained by these organic wastes that serve as a possible food source at the water-sediment interface. At the innermost part of Osaka Bay, where the bottom currents are weak, a large amount of organic debris seals in the sediment. That organic matter apparently nourishes a large population of Capitelia species. Such a difference in the form of organic matter may lead to the success of distinct dominant species in these two localities.

SUMMARY AND CONCLUSIONS

Community structure and seasonal fluctuations of the macrobenthos around the fish farms in Gokasho Bay are similar in some respects to those in Osaka Bay, which is polluted by sewage wastes, but are different from those in Omura Bay, which is characterized by an enclosed topography. High organic input from the fish farms in Gokasho Bay eliminates the macrofauna during the summer, but induces an overwhelming dominance by the suspension and surface deposit feeder *P. paucibranchiara* in the oxygen-recovery season. Environmental deterioration around the fish farms is obvious, but is not usually as severe as it is in Osaka Bay, where *Capitella* sp. dominates. Thus, we can monitor the bottom environments around the fish farms by examining the community structure, seasonal fluctuations, and species composition of the macrobenthos.

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Nitrogen Budget and Water Quality Management in Larviculture Ponds of the Swimming Crab

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ABSTRACT

The objectives of the present study are to determine the cycle and budget of nitrogen in larviculture ponds of the swimming crab *Portunus trituberculatus* and to evaluate their impact on water quality and survival rate. Field surveys were carried out at the Hiroshima Prefecture Fish Farming Center from 1990 to 1993. Among nitrogenous compounds. concentrations of NH_4-N and particulate organic nitrogen (PON) showed peaks just before the mass exchange of pond water, when the main component of sedimentary material changed from phytoplanktonic organic matter to nonphytoplanktonic organic matter. As a result of N budget analysis, it was made clear that the main factors enhancing N concentration in the pond are the input of phytoplankton culture medium and feed in Phase I, while only feed contributed in Phase II. High survival rate is supported both by low input and low concentration of N, especially of NH_4-N, when high conversion rate of feed-N to crab larvae-N was attained. Then, variation in the individual nitrogenous compound per unit time per pond (gNd^{-1} pond $^{-1}$) revealed that feed, sedimentation and water discharge are the main factors affecting the variation of NH_4-N. Input of concomitant NH_4-N in "green water" (culture of green algae) also significantly contributed to the increase of NH_4-N concentration in the pond. As practical measures for improving the culture system, reduction of phytoplankton load and feeding load to the level which causes NH_4-N concentration below 34 μg at l^{-1} are proposed. These measures will improve not only the survival rate and the cost of crab seed production but also the water quality of pond water. Thus, the environmental impact of effluent water will be significantly minimized.

INTRODUCTION

Larviculture of the swimming crab ***Portunus trituberculatus*** has been extensively carried out in Japan in order to improve the natural resources of the crab in the coastal area. However, the survival rate during the larviculture for seed production is unstable and sometimes extremely low because of high mortality due to unknown reasons but which are supposedly due to water quality management. Among many parameters used to characterize the water quality, N is one of the most important indicators of water quality because N is not only a major chemical component of the organism but also a major component of the feed supplied and excrement of the crab larvae. Although acute toxicity of ammonia and nitrite on crustaceans has been studied, the chronic effect of nitrogenous compounds on larvae of the swimming crab is not clear (Chen and Chin 1988, Chen et ***al.*** 1990). From the viewpoint of seed production, quantitative evaluation of water quality management through nitrogen budget analysis is very important to improve the survival rate. Hence, the objectives of the present study are to make clear the cycle and budget of nitrogen through field observation in larviculture ponds of the swimming crab and to evaluate their impact on water quality and survival rate.

MATERIALS AND METHODS

Field surveys were carried out at the Hiroshima Prefecture Fish Farming Center in Takehara, Japan, every year from 1990 to 1993 on the substantial crab seed production. During this time, crab larvae were metamorphosed from the stage of zoea 1 followed by zoeae 2, 3, 4 and megalopa into crab 1. According to the stage of crab larvae, methods of the culture management changed (Table 1). During the stage of zoea 1 to early zoea 3, water exchange rate was very small but mass exchange of water was carried out after the latter stage of zoea 3. When the zoea attained stage 4, sediment accumulated on the concrete pond floor was removed in order to prevent the unfavorable effect of sediment on the water quality.

From the viewpoint of water quality management, we divided the culture period into Phase I (before sediment removal) and Phase II (after sediment removal) in the present study. During Phase I, phytoplankton culture was supplied as empirical measures to maintain water quality.

Table 1. Water management of crab (*Portunus trituberculatus*) larvae rearing pond and feed supplied in tbe present study

Phase	I				II	
Larval stage	Zoea 1	Zoea 2	Zoea 3	Zoea 4	Megalopa	crab1
Water volume (m^3 pond-')	40-65	65-85	85	85	85	85
Water exchange (m^3 d^{-1} $pond^{-1}$)	O-10	o-51	30-100	70-500	70-500	70-500
Feed						
B. plicatilis (108 ind. pond-')	4-10	6-20	7-35	8-55		
A. salina nauplii (108 ind. pond-')		0.2-1.o	0.2-2.5	0.4-4.5	0.5-6.0	
Minced shrimp (kg $pond^{-1}$)					0.25-4.5	
Formula feed (gm $pond^{-1}$)	100		100-400	100-600	200-800	

Depending upon the phytoplankton culture, the pond water was classified as G: green water (green algae), B: brown water (diatom) and Bffi: mixture of green and brown waters. Nitrogen source of the cultured phytoplankton was ammonium sulfate [(NH&SO,] and potassium nitrate (KNO_3) for green water and brown water, respectively. Feed also changed according to larval stage (Table 1). During the stage of zoea 1 to zoea 4, rotifer, brineshrimp larvae and artificial feed were supplied, while, during the stage from megalopa to crab 1, brineshrimp larvae, minced mysis and artificial feed were supplied. Initial number of larvae (zoea 1) introduced into the ponds ranged from 1,230,000 to 2,360,000/pond, while final yield of seed production ranged from 43,000 to 622,000 (crab 1 larvae)/ pond depending on survival rate.

Analyses of ammonium nitrogen (NH_4-N), nitrite-N (NO_2-N) and nitrate-N (NO_3-N) were made according to the manual of the Oceanographical Society of Japan (1990). Particulate organic nitrogen (PON) was measured by a CHN analyzer (Yanaco MT-3, Yanagimoto Co.). Determination of chlorophyll a (Chl a) in pond water was made by the method of Strickland and Parsons (1972). PON and Chl a of sediment were similarly analyzed as those in pond water. Nitrogen content of feed, phytoplankton culture and crab larvae were also determined by the CHN analyzer in order to calculate the N budget.

RESULTS AND DISCUSSION

VARIATION OF NITROGEN COMPOUNDS IN POND WATER DURING LARVICULTURE

Among nitrogenous compounds, NH_4-N showed a peak just before the mass exchange of pond water, especially in the green water pond (Fig. 1). NH_4-N concentration was generally high in the green water pond, ranging from 2.1 to 237 μg at l^{-1}, compared with the brown water pond in which the values ranged from 0.1 to 30 μg at l- 1. Not only NH_4-N but also NO,-N showed higher concentration in the green waterpond compared with the brow nwater pond, and their concentrations ranged from 0.0 to 9.0 μg at l^{-1}. Variation of NO,-N concentration, however, was affected by fertilization, showing th emaximum of 46 μg at l^{-1}. The 24-h median lethal concentrations of NH_4-N, NO,-N and NO,-N for each larval stage of the swimming crab are reported to be 4,800-7,600, 14,000-26,500 and 160,000 280,000 μg at l^{-1}, respectively, and are lower for molting larvae from zoea 1 to zoea 2 (Mawatari and Hirayama 1975). From these observations, it is suggested that acute toxicity of observed N compounds is not significant.

In order to estimate the contribution of phytoplanktonic N in the PON, PON:Chl a ratio was examined. PON:Chl *a ratios* were below 70 both in suspended matter and in sediment in Phase I, while in Phase II these ratios were significantly higher than Phase I (Fig. 2). Assuming that PON:Chl *a* ratio of phytoplankton is less than 16 (Parsons et al. 1984). it is suggested that the main component of suspended matter and sediment was phytoplanktonic organic matter in Phase I, while in Phase II it changed to nonphytoplanktonic organic matter.

ESTIMATION OF NITROGEN BUDGET DURING LARVICULTURE

In order to determine the N budget in Phase I and Phase II, each component of N input into the pond, such asini

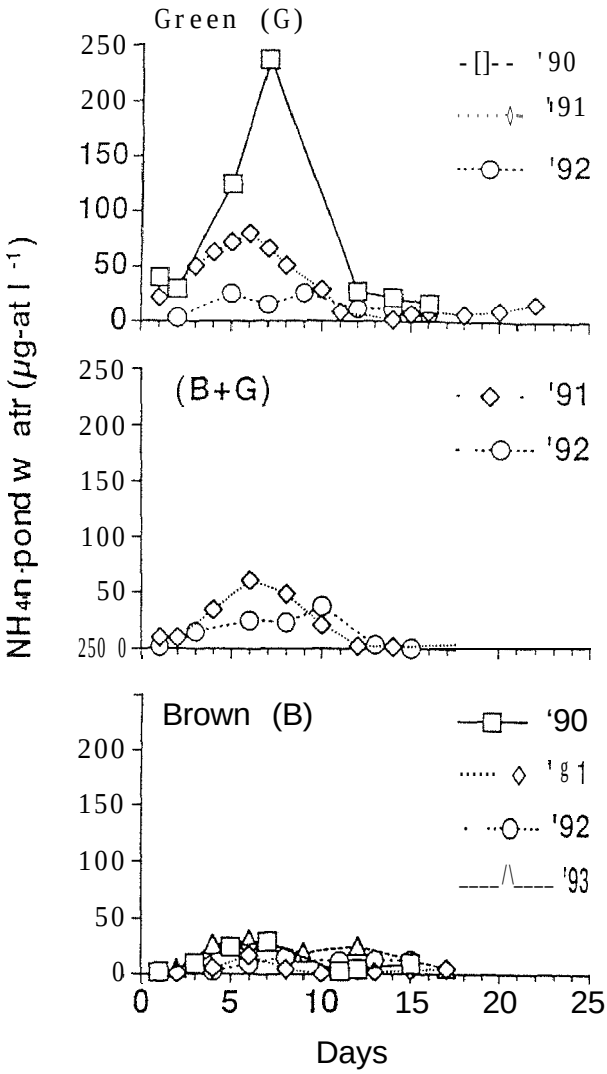


Fig. 1. Variation in ammonium-N concentration in three different kins of pond water from 1990 to 1993.

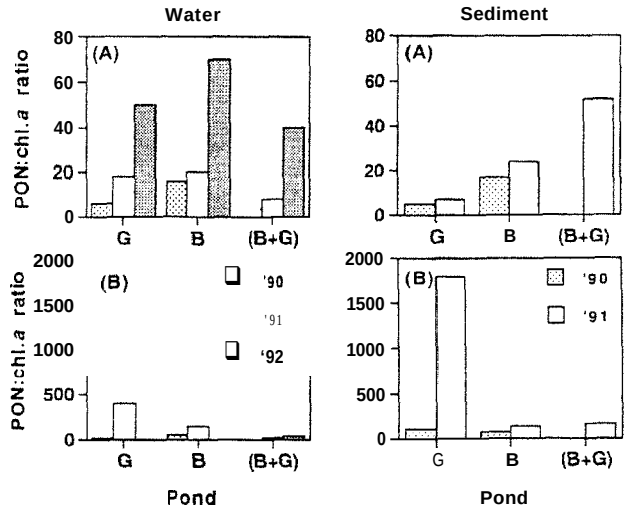


Fig. 2. Average PON:Chl a ratio in waters and sediments of three different kinds of ponds. (A) phase I, and (B) phase II. Two ponds, green water and brown water where the sediments were not removed during the rearing period in 1992, were also included.

tial introduction of larvae, feed supply, phytoplankton sup ply and seawater inflow as well as each component of N output, such as crab larvae, sediment and outflow of seawater, were estimated (Table 2). In Table 2, percent composition of input and output N is also presented. As a result of these N budget analyses, it was made clear that the main factors contributing to the high level of N in the pond were the input of phytoplankton culture medium and feed in Phase I, and only feed in Phase II.

Transformation or transportation rate of nitrogenous compound per unit time per pond (gNd^{-1} pond-') on the individual pathway of N cycle in the larviculture was estimated. These results revealed that sedimentation, phytoplankton supply and water discharge were the main factors affecting the variation of N in the green water pond, while feeding, sedimentation and water discharge were the main controlling factors of N in the brown water pond (Fig. 3).

CLASSIFICATION OF NITROGEN LEVEL

As factors indicated the N level of the larviculture system, NH_4-N concentration in the pond water in Phase I and Phase II of larviculture were classified into low (L), medium (M) and high (H) levels. In this classification, L indicates the values lower than 1/3 of the maximum values, M indicates the values between 1/3 and 2/3 of the maximum, and H indicates the values higher than 2/3 of the maximum. For NH_4-N concentration, the borders of L and M and M and H were 17 and 34 μg at l^{-1} for Phases I and VII and 14 μg at l^{-1} for Phase If.

Since NH_4-N was contained in the culture media of phytoplankton especially in the media used for green water, carryover of media NH_4-N to the pond was calculated. As a result, a small proportion of NH_4-N was estimated to originate from culture media used in the green water pond.

RELATIONSHIP BETWEEN NITROGEN LEVEL, SURVIVAL RATE AND LARVAL PRODUCTION

Since survival rate is closely related to final seed production (Fig. 4), for practical use we can evaluate the final performance of larviculture by the level of survival rate. The survival rate (SR) was classified into four ranks, from rank A to rank D, based on the survival rate observed in the present study: rank A of SR ≥30%, rank B of 20% ≤ SR < 30%, rank C of 10% ≤ SR < 20% and rank D of SR < 10%.

Relationships between NH_4-N level and rank of larval production indicated by survival rate are presented in Table 3. Rank A of larval production was established only when

Table 2. Nitrogen budget in crab larvae reating ponds during (a) Phase I and (b) Phase II. Percentage values are in parantheses. (a)

Pond Year	Input [gN] Larvae	Feed	Phytoplankton	W. Inflow	Total	Output [gN] Larvae+R.[a]	Sediment	W. Outflow	Total
Green									
1990	2 (<1)	282 (19)	1130 (78)	43 (3)	1460 (100)	99 (7)	866 (59)	492 (39)	1460 (100)
1991	2 (<1)	310 (32)	426 (44)	229 (24)	967 (100)	108 (11)	209 (22)	650 (67)	967 (100)
Brown									
1990	1 (<1)	316 (60)	162 (31)	45 (9)	524 (100)	25 (5)	175 (33)	324 (62)	524 (100)
1991	2 (<1)	291 (62)	18 (4)	159 (34)	469 (100)	25 (5)	73 (17)	371 (79)	469 (100)
1993	2 (<1)	883 (83)	2 (<1)	177 (17)	1060 (100)	64 (6)	169 (IS)	831 (78)	1060 (100)
B+G									
1990	2 (<1)	364 (25)	471 (32)	626 (43)	1460 (100)	60 (4)	209 (14)	1190 (82)	1460 (100)
1991	1 (<1)	1060 (77)	119 (9)	191 (14)	I370 (100)	81 (6)	216 (18)	1070 (78)	1370(100)

(b)

	Larvae+R	Feed	Phytoplankton	W. Inflow	Total	Crab	Sediment	W. Outflow	Total
Green									
1990	99 (4)	1560 (65)	481 (20)	270 (11)	2410 (100)	19 (1)	1420 (59)	968 (40)	2410 (100)
1991	108 (8)	952 (69)	0 (0)	322 (23)	1380 (100)	7 (1)	841 (61)	534 (39)	1380 (100)
Brown									
1990	25 (1)	1580 (80)	86 (4)	272 (14)	1960 (100)	56 (3)	906 (46)	998 (51)	1960 (100)
1991	25 (2)	652 (42)	16 (1)	863 (56)	1560 (100)	71 (5)	237 (15)	1250 (80)	1560 (100)
1993	64 (17)	244 (66)	0 (0)	60 (16)	367 (100)	17 (5)	74 (20)	277 (75)	367 (100)
B+G									
1990	60 (5)	844 (66)	28 (2)	345 (27)	1280 (100)	60 (5)	262 (21)	954 (75)	1280 (100)
1991	81 (10)	405 (49)	29 (4)	306 (37)	821 (100)	3 (<1)	158 (19)	660 (SO)	821 (100)

[a] Larvae and residues in water.

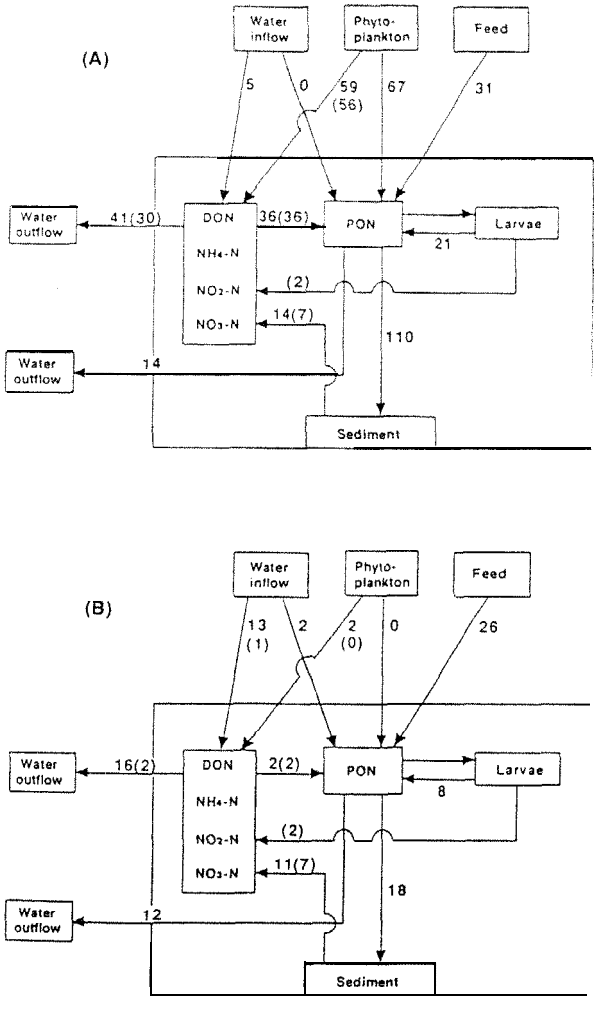


Fig. 3. Schematic presentation of the example of estimated N flow (gND^{-1} pond'), (A) in the green water pond (1990), and(B) in the brown water pond (1991).

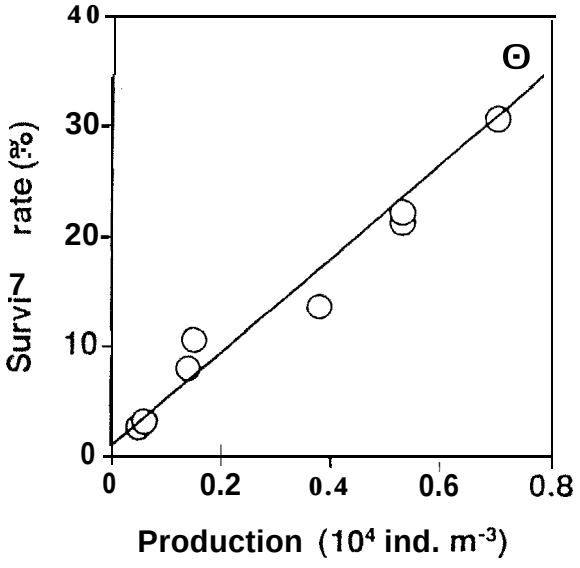


Fig. 4. Relationship between production and survival of swimming crab larvae in different kinds of ponds from 1990 to 1993.

Table 3. Relationship between NH_4-N level and production of swimming crab larvae. Number shows the case observed.

NH_4-N Level	Rank of Production			
	A	B	C	D
Phase I				
H			1	2
M		1		1
L	1	1		
Phase II				
H			1	
M				2
L	1	2		1

NH_4-N level was low in Phases I and II. Rank B of larval production was always associated with lower medium NH_4-N level, while rank C or D was sometimes associated with high NH_4-N level. From these results, it is concluded that high larval production or high survival rate is supported by low NH_4-N concentration. Since no acute toxicity by observed NH_4-N concentration is suggested, the present result means that NH_4-N concentration may be an indicator of water quality representative of the individual larviculture management. For more detail, we can indicate the recommended NH_4-N concentration by the method designating the NH_4-N concentration which enabled the production of ranks A and B. By the same method, we can realize the favorable N input and output levels in Phases I and II. From the result of Phase I. it can be estimated that the case of maximum input is three times higher than recommended input and also excess feeding was suggested from the percent composition of input N (Table 2) in some cases (Fig. 5a). On the other hand, in the case of Phase II, although no excess feeding was observed, low survival rate was observed in some cases (Fig. 5b).

ORIGIN OF SEDIMENT NITROGEN

The origin of sediment N was estimated by the content of Chl *a* under the assumption that phytoplankton N:Ch.l a ratio was constant at 16 (Fig. 6). From these ratios, the main component of sediment N in the green water pond in Phase I was suggested to be sedimented green algae. On the other hand, nonphytoplanktonic N was suggested to be the main component of sediment after the mass exchange of water during which no phytoplankton culture

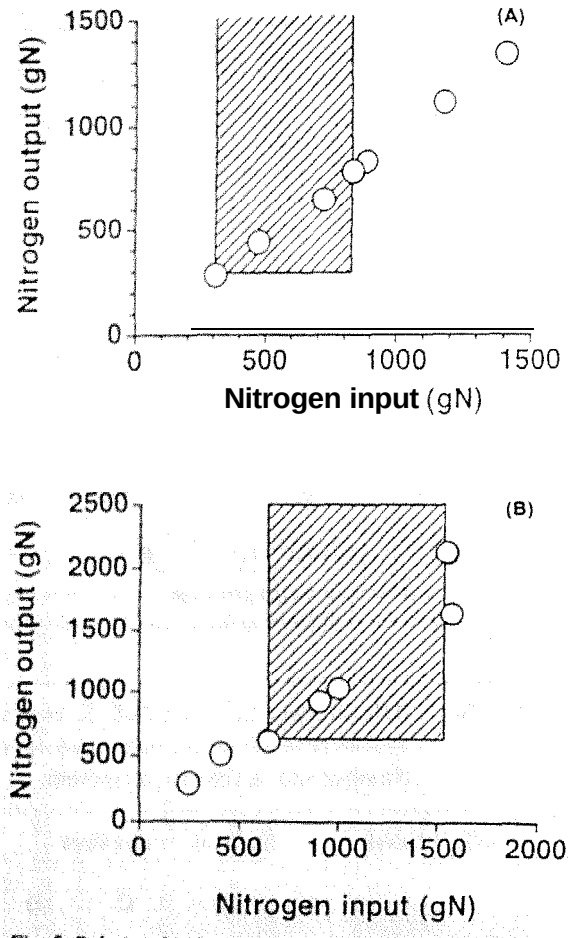


Fig. 5. Relationship between the nitrogen input and the nitrogen output. (A) phase I, and (B) phase II. Recommended levels of nitrogen input and output are indicated by hatch based on survival ranking (A and B).

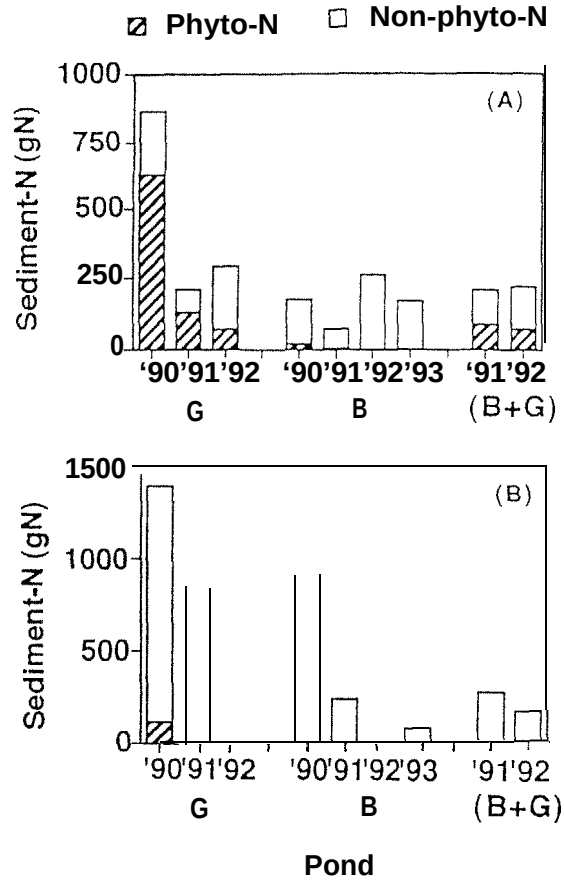
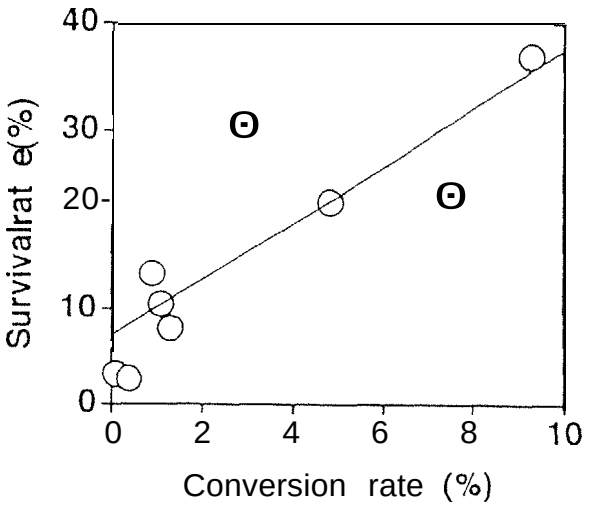


Fig. 6. Amount of nitrogen in sediment contributed by phytoplankton and nonphytoplankton sources. The amount of nitrogen by phytoplankton source was estimated by Chl a content assuming the N:Chl a ratio is constant at 16.



Relation between conversion rate and survival rate

Fig. 7. Relationship between conversion rate and survival rate. Conversion rate was calculated as percentage of larvae-N increased to feed-N supplied during the whole rearing period.

was supplied. The sediment in Phase II is supposed to be feces, unconsumed feed and dead bodies of organisms.

RELATION BETWEEN CONVERSION RATE OR SURVIVAL RATE AND WATER QUALITY

We calculated the conversion rate of feeding N supplied to crab larvae-N harvested (Fig. 7). From these results, it can be concluded that the high survival rate which generally means high production is associated with the high conversion rate. When the conversion rate was low, nitrogen that was not converted to crab N might have deteriorated the water quality, while the high conversion rate might have contributed not only to the improvement of water quality but also to the cost performance of seed production.

CONCLUSION

High NH_4-N concentration in the culture pond generally indicated low survival rate of crab larvae. Less than 34 $\mu g\text{-at } l^{-1}$ of NH_4-N concentration is recommended for improving larviculture. In order to reduce NH_4-N concentration, reduction of N input, especially reduction of phytoplankton load and feeding load is recommended. As to the N source of the phytoplankton culture media, NO_3-N is preferable compared to NH_4-N. These measures will improve not only the survival rate and the cost of crab seed production but also the environmental impact of effluent water.

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Shrimp Farms' Effluent Waters, Environmental Impact and Potential Treatment Methods

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ABSTRACT

Texas has a *2280 km (1,425 mile)* coastline and vast amount of coastal land which is not suitable for traditional agriculture crops. This land can be used for the development of a shrimp farming industry with an estimated value of $100 million or greater within the next 10 yr. However, this industry will face restrictions from regulatory agencies that will limit future growth and may even reduce the present production level of shrimp in Texas. The concern of the agencies lies with the emission of effluent water generated by shrimp farms. In an effort to reduce the potential negative impact on coastal waters, current regulation by the Texas Natural Resources Conservation Commission (TNRCC) requires effluent water from shrimp farms to meet standards set for municipal and industrial wastewaters. Preliminary effluent characterization of three farms in south Texas suggests that in two farms, the total suspended solids (TSS) and ammonia (NH_4-N) levels were higher than the standards set by TNRCC. The TSS and five-day carbonaceous biochemical oxygen demand (CBOD,) for the third farm were higher than the required standards. Coagulation methods, although effective in decreasing inorganic effluent TSS level, were cost-prohibitive and not adequate for ammonia and algal removal. A research team from Texas Agricultural Experiment Station, Texas Agricultural Extension Service, Texas A&M University-Kingsville (TAMU-Kingsville), Texas A&M University-Corpus Christi and The University of Texas-San Antonio is currently working with the shrimp producers to evaluate potential methods to improve effluent water quality. Studies were initiated to develop alternative feeding and pond management practices including reduction in pond water exchange rates. Development of a low protein, low pollution diet with higher nitrogen and phosphorus digestibility is another promising option to decrease effluent nutrient loads. Circulating effluent waters via settling basins, bivalves and seaweed beds, and constructed wetlands are another potential alternative to give the shrimp farmers cost-effective effluent treatment methods. Due to the recent Taura virus disease outbreak in south Texas, only initial evaluation of the above effluent treatment strategies was carried out during the 1995 season.

INTRODUCTION

Aquaculture, the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants, is increasing worldwide. Since 1984 there has been a consistent growth in aquaculture production and the distribution of its products. Aquaculture production in 1990 constituted approximately 15.3% of the world's fishery production (FAO 1992) as compared to 14% in 1989 (New 1991). From 1984 to 1992 there was an 89% increase in shrimp production. The reported annual production for 1994 was 20.8 million metric tons (Mmt). Based upon population growth, in the yr 2000 there will be 6.3 billion people with per capita consumption of 19 kg of whole fish. This statistic will necessitate a production quota of 120 Mmt. The contribution from fisheries for 1990 was about 84.6 Mmt. Assuming contribution from this source will stay at the same level, the aquaculture industry will have a production need of 35 Mmt by the year 2000 (Gallagher and Gallagher 1995). This same trend is forecasted for the shrimp farming industry. Over a decade and a half ago, this industry provided only 5% of the total shrimp placed on the world market compared to the 25% being produced in 1994 (Rosenberry 1994).

Aquaculture by definition uses resources from and interacts with the environment. Barg (1992) claimed that the majority of aquaculture practices have had little adverse effect on ecosystems. Generally, the expansion of aquaculture results in the provision of food, increased income, employment and foreign exchange earnings (Schmidt 1982, Pullin 1989, Pillay 1992). Furthermore, stocking and release of hatchery-reared organisms into inland and coastal waters can also help alleviate depletion of wild fisheries stocks. Culture of molluscs and seaweeds may in some cases reduce nutrient and organic enrichment in

eutrophic waters. On the other hand, productivity of oligotrophic waters may be enhanced due to the nutrient and organic wastes released from aquaculture farms (FAO 1992). Nevertheless, some cases of environmental degradation in coastal areas have been documented due to intensive aquaculture operations in Europe and shrimp farming activity in Southeast Asia and Latin America (Phillips *et al.* 1993). Many aquaculture operations release metabolic waste products such as feces, ammonia and uneaten food into the receiving waters. In most cases, the organic particulate waste will accumulate on the seabed in the immediate vicinity of the farm, while the soluble waste *will* eventually end up in the receiving waters. Organic enrichment of the benthic ecosystem may result in an increased oxygen consumption by sediment communities and the formation of anoxic conditions. There is evidence of very localized effects of reduced concentrations of dissolved oxygen in bottom and surface waters close to aquaculture sites, The reductions are due to the considerable biochemical oxygen demand of released organic wastes and the respiratory demands of the cultured stock. In extreme cases, production of carbon dioxide, methane and hydrogen sulfide, followed by reduction in macrofauna biomass, abundance and species composition, may also follow. At the same time, the increase in soluble inorganic nutrients (nitrogen and phosphorus species) can result in an increase in primary productivity of microorganisms in the receiving waters (Barg 1992).

Several tropical countries have lost extensive mangrove areas due to clearing and conversion of the land to fish and shrimp ponds (Phillips *et al.* 1993). The clearing operations are often accompanied by salinization and acidification of soils and aquifers. These areas are important breeding and nursery grounds for many commercially exploited fish and shellfish species. Although large areas of mangrove swamps have been cleared for shrimp pond construction (Lin 1995), it is important not to underscore the fact that mangrove ecosystems have also been utilized for other purposes. including forestry, agriculture and fishfarming (Neal 1984, FAO 1985, Andriawan and Jhamtani 1989, Soemodihardjo and Soerianegara 1989). It is clear that uncontrolled development of the aquaculture industry can have devastating effects on coastal ecosystems These include (1) destruction or degradation of wetland and mangrove habitats; (2) eutrophication of receiving waters; (3) pollution of receiving waters from chemicals and amendments added to pond water; (4) excessive organic loading of substratum and alteration of benthic communities; (5) overuse of limited water resources; (6) salination of soils and coastal aquifers; (7) overfishing of wild stocks to provide seed and brood stock; (8) capture and destruction of estuarine biota, (9) introduction of non-native species and pathogens; (IO) interbreeding between cultured and wild populations; and (1 I) displacement of traditional coastal communities.

When dealing with environmental impact of aquaculture, it is useful to distinguish between extensive, semi-intensive and intensive farming systems. Under extensive management systems, cultured organisms are kept at low densities and may occasionally receive additional nutrition through fertilization. In semi-intensive aquaculture, cultured organisms are kept at higher densities than in *extensive* systems. The culture media are often fertilized and supplementary feed may be provided. On the other hand, in intensive production systems, cultured organisms are kept at high densities and prepared feed is provided regularly.

In semi-intensive and intensive pond systems, it is not uncommon to have up to a 30-40% pond water volume exchange a day to supply oxygen and to improve water quality. In Taiwan, shrimp farms' water requirements are reported to vary between 11,000 and 2 1,430 m^3 for every ton of shrimp produced in semi-intensive culture systems and between 29,000 and 43,000 m^3/t for intensive culture operations (Chien et *al.* 1989). Hopkins and Villalon (1992) found only small correlation between estimated water usage per unit weight of product and shrimp production rates. Often on large farms, water exchange is based on a set schedule, with occasional emergency flushes (Macia 1983), rather than as an ongoing response to changing pond conditions. Water exchange rates are seldom based on well-conceived nutrient and algal population monitoring. Hopkins *et al.* (1995a) reported undocumented cases where water exchange had been used to flush phytoplankton blooms in response to increased ammonia levels or low dissolved oxygen which was found to be counter-productive. Often, pond flushing removes phytoplankton and nitrifying bacteria that could have otherwise improved pond water quality. Furthermore, flushing does not usually affect metabolic processes on the pond bottom, a site where ammonia being produced and oxygen is being consumed. In a well balanced pond system, plankton and bacteria populations can have a positive long-term stabilizing effect on pond water quality. Hopkins *et al.* (1993) studied the effect of water exchange rates on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. They reported that reducing typical water exchange rates in intensive ponds is feasible without negatively affecting shrimp survival or growth, thereby decreasing economic costs and the potential environmental impact of effluents. Hopkins and co-workers (1995b) mentioned that high shrimp production can be achieved without water exchange (7,000kg/ha/crop). To avoid nutrient release into receiving waters during harvest, they suggest storing it for reuse with subsequent crops. While the idea of water recycling systems is ecologically sound, the efficiency of the system is still far from being perfected.

Table 1. Effluent characteristics and monitoring requirements **based on water discharge permit issued by Texas Natural Resource Conservation Commission (TNRCC) for Taiwan Shrimp Village Association farm in south Texas.**

Parameter	Daily average	Daily min.	Daily max.	Single grab	Frequency and sample type
Discharge (m_3/day) (MGD)	378,540	N/A	681,372	N/A	1/day, continuous
		100		180	
pH	N/A	6.0	9.0	N/A	1/day
DO (mg/L)	6.0	3.0	N/A	N/A	3/day, av.
NH_4-N (mg/L)	1.0	N/A	2.0	3.0	3/wk, composite
$CBOD_5$[a] (kg/day) (lb/day)	1.513 3,336	N/A	2,268 5,000	N/A	3/wk, composite
$CBOD_5$ (mg/L)	4.0	N/A	6.0	8.0	3/wk, composite
TSS[b] (mg/L)	30	N/A	Report	N/A	3/wk, composite
TSS[c] (mg/L)	15	N/A	30	50	3/wk, composite

a Five-day carbonaceous biochemical oxygen demand
b Total suspended solids
c Net increase over intake level based on Water Discharge Permit for Southern Star Inc.

Feed is the major source of nutrient and particulate loads in aquaculture effluent (Avnimelich and Lacher 1979, Krom and Neori 1989). Nitrogen and phosphorus pollution from feeds in effluent water were identified as a major concern to the receiving waters (Kaushik and Cowey 1991, Lin 1995). In Japan, Canada and some Scandinavian countries, the concern for pollution by aquaculture feeds has resulted in regulations governing major feed components. These regulations sometimes result in limited animal growth (Jensen 1991). Since shrimp are bottom feeders, their feed consumption is difficult to estimate, and overfeeding, the main cause for organic loading and pond bottom deterioration, often follows. Use of feeding trays is an important to01 to evaluate shrimp feed consumption and to adjust feeding rates accordingly. Moya (1993) reported the results of growout trials conducted with *Penaeus vannamei* in Peru and Honduras using feed trays in 23 ponds. A significant reduction in feed conversion ratios (FCR) was obtained (between 0.9 and 1.3) for 57% of the ponds tested. This reduction in FCRs can also be obtained by including highly digestible protein sources and well balanced amino acids in the diet. Cho and co-workers (1994) developed low pollution diets using nutrient-dense formulations to achieve very low FCRs (≈ 1: 1). Decreasing protein level in the diet is another promising solution to reduce nutrient load in shrimp farms' effluent waters. Aranyakananda and Lawrence (1993) found that by increasing feeding frequency, the protein level of *P. vannamei can be* greatly reduced (from 35 to 15%), without negatively affecting growth and survival. Villalon (1991) suggests that increasing the feeding frequency should have an immediate benefit, including reduced nutrient leaching and feed loss, increased growth, and feed use efficiency. Promising results were recently achieved through the use of 20% protein feeds vs. 40% protein diets with no water exchange in a trial in South Carolina (Chemberlain and Hopkins 1994, Hopkins *et al,* 1995b). It has been speculated that under these conditions, bacterial use of waste feed is stimulated since the lower protein levels offer more optimal carbon to nitrogen ratio (Avnimelech et *al.* 1992, Kochba et al. 1994). Initial results with this approach in a small-scale experiment show a 50% reduction of feed cost for both shrimp and tilapia. Environmental impact is simultaneously reduced through decreased water exchange. Research is currently being devoted to improving the long-term viability of marine shrimp farming. Several areas have been emphasized including proper site selection, prevention of escapement, control of disease, captive breeding of healthy genetically improved stocks, and better system designs and management protocols.

Shrimp production from Texas farms was less than $2 million in 1990, with shrimp becoming the most valuable aquaculture crop in Texas in 1992 (Jensen 1993). With the 2,280 km (1,425 mile) coastline and the availability of coastal land that is not adequate for traditional agricultural crops, shrimp farming in Texas could have a value of more than $100 million within 10 yr. The effluent waters

Table 2. Changes in water quality parameters aver a 24-h period in selected sampling stations on and near **Southern** Star Inc. (SSI) and Tafwan Shrimp Viiage Association (TSVA) farms.

Date	Time	Parameter(mg/l)	TV1a	TV2b	TV3c	MDCd	CDCe	SS1f	SS2g
9-30-94	18:00	TSS[h]	23	22	104	134	22	10	112
9/30	22:00		14	48	72	N/A	42	32	68
10/1	2:00		11	32	26	28	148	21	52
10/1	7:00		27	44	26	138	114	24	20
10/1	11:00		18	30	56	32	80	9	88
10/1	14:00		13	94	82	136	246	21	112
9-30-94	22:00	$CBOD_5$	2	2.4	2.5	1.8	2.6	N/A	N/A
10/1	2:00		N/A	N/A	N/A	N/A	N/A	2.7	1.8
10/1	7:00		1.6	0.9	2.6	1.4	8.8	2.6	2.2
10/1	14:00		2.7	2.2	2.2	2.7	5.6	4	1.4
9-30-94	18:00	Tp[i]	0.2	0.45	0.6	0.46	0.11	0.27	0.29
9/30	22:00		0.2	0.43	0.38	0.37	0.13	0.26	0.22
10/1	2:00		0.21	0.36	0.37	0.38	0.16	0.22	0.18
10/1	7:00		0.24	0.35	0.35	0.34	0.16	0.3	0.23
10/1	11:00		0.21	0.32	0.27	0.38	0.53	0.23	0.19
10/1	14:00		0.22	0.42	0.35	0.44	0.36	0.23	0.28
9-30-94	18:00	RP[j]	0.18	0.35	0.44	0.33	0.03	0.16	0.22
9/30	22:00		0.15	0.33	0.3	0.31	0.02	0.19	0.13
10/1	2:00		0.13	0.3	0.27	0.28	0.03	0.15	0.14
10/1	7:00		0.19	0.33	0.27	0.3	0.03	0.19	0.2
10/1	11:00		0.18	0.31	0.25	0.33	0.46	0.18	0.19
10/1	14:00		0.1	0.2	0.15	0.25	0.33	0.15	0.09
9-30-94	18:00	NH_3-N	0.54	1.04	1.4	1.93	0.01	0.46	0.3
9/30	22:00		0.6	1.52	1.28	1.32	0.05	0.72	0.33
10/1	2:00		0.68	1.72	1.28	1.28	0.03	0.53	0.48
10/1	7:00		0.68	1.52	1	1.32	0.01	1.04	1.04
10/1	11:00		0.36	1.32	0.84	1.24	2.2	0.84	0.44
10/1	14:00		0.29	1.44	1.12	1.48	1.32	0.28	0.6
9-30-94	18:00	NO_3-N	0.3	0.6	0.5	0.5	0.2	0.3	0.2
9/30	22:00		0.3	0.7	0.4	0.5	0.3	0.3	0.3
10/1	2:00		0.3	0.6	0.4	0.5	0.2	0.3	0.4
10/1	7:00		0.3	0.6	0.4	0.5	0.3	0.5	0.3
10/1	11:00		0.4	0.6	0.3	0.6	0.4	0.5	0.3
10/1	14:00		0.4	0.8	0.4	0.6	0.4	0.5	0.3
9-30-94	18:00	NO_2-N	0	0.35	0.26	0.3	0.04	0.07	0.08
9/30	22:00		0.08	0.37	0.16	0.27	0.07	0.07	0.09
10/1	2:00		0.07	0.37	0.16	0.32	0.03	0.12	0.17
10/1	7:00		0.05	0.36	0.14	0.28	0.03	0.07	0.08
10/1	11:00		0.07	0.39	0.13	0.32	0.23	0.1	0.12
10/1	14:00		0.09	0.4	0.14	0.36	0.14	0.09	0.12

a TWA intake station
b TSVA outlet collecting effluent water from ponds #26 through #66
c TSVA outlet collecting effluent water from ponds #1 through #25
d Main drainage canal station with effluent contribution from SSI, TSVA, and county drainage canal (CDC)
e Station at the CDC
f SSI intake station
g SSI farm's effluent discharge station
h Total suspended solids
I Total phosphorus
j Reactive phosphorus

Table 3. Changes in total suspended solids (TSS) levels in different stations along the **Arroyo** Colorado during the 1994 preharvest season.

Date	TSS[a] (mg/L)								
	400 m upstream			Discharges			400 m downstream		
	0.3[a]	0.9	2	0.3	0.9	1.5	0.3	0.9	1.5
7/19/94	42	42	83	172	68	38	19	23	45
7/26/94	11	15	17	23	9	15	9	6	22
8/16/94	42	N/A	79	220	N/A	82	12	N/A	22
	800 m downstream			1200 m downstream			1600 m downstream		
	0.3[b]	0.9	2	0.3	0.9	1.5	0.3	0.9	1.5
17/19/94	20	20	47	48	13	34	29	15	28
7/26/94	6	21	16	15	16	14	8	9	10
8/16/94	12	N/A	24	18	N/A	10	10	N/A	14

[a]TSS

[b]Water depth (in meters) from which samples were taken

generated by the shrimp farms currently create a serious growth limiting factor for the emerging Texas shrimp farming industry. It is particularly true for Texas coastal areas, in which the discharge is going into bays and estuaries behind barrier islands that have limited water exchange with the Gulf of Mexico. Small warm-water aquaculture facilities that discharge less than 30 days/yr and with harvest of 45,000 kg/yr (100,000 lb) or less are generally exempt from having a Water Discharge Permit. The Texas Natural Resource Conservation Commission (TNRCC) requires that any large aquaculture facility that discharges large volumes of water will have a Water Discharge Permit. Currently, there are only two shrimp farms in the state that carry this permit, the Southern Star Inc. (SSI) and Taiwan Shrimp Village Association (TSVA). The SSI is the only farm currently required to monitor continuously the flow and effluent volume being discharged into the river. Compliance of the other farm with daily averages and maximum water discharge limits is based on daily flow measurements. In addition, the farms are required to monitor total suspended solids (TSS), dissolved oxygen (DO), pH, ammonia (NH_4-N), and five-day carbonaceous biochemical oxygen demand ($CBOD_5$). Table 1 provides a summary of the monitoring requirements for these two farms. The table lists only the parameters for which the TNRCC has set limits. Other parameters such as fecal coliform bacteria, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total phosphorus, salinity, volatile suspended solids (VSS) and chlorophyll a are required to be monitored for reporting purposes only. Meeting effluent water quality standards is a vital factor for the survival of the shrimp farming industry in Texas. To ensure continuous growth of this industry, the Texas Agricultural Experiment Station (TAES) initiated a study to characterize and develop cost-effective management and treatment strategies for effluent waters of shrimp farms. The specific objectives of this study were as follows: (1) characterize the effluent water of the three farms; (2) monitor the impact of effluent TSS from two farms on the receiving waters; (3) determine whether soil erosion of earthen drainage canals contributes significantly to the high TSS levels observed in shrimp farms' effluent water; and (4) suggest a cost-effective treatment strategy to improve effluent water quality of the farms. In an effort to meet these goals, selected water quality parameters of the farms' intake and effluent waters were monitored for four months to cover the harvest season. Samples from receiving water of SSI and TSVA farms were also analyzed to evaluate the effect on the environment. Water samples were analyzed either at the water quality laboratory on the farms' site (VWTL) or at Texas A&M University-Kingsville, using EPA and standard methods procedures (API-LA et al. 1992).

SAMPLING OF RECEIVING WATERS OF TWO AQUACULTURE FACILITIES ON THE ARROYO COLORADO

Several short-term intensive sampling studies were conducted during the preharvest and the harvest seasons of two farms. These studies were designed to understand the diurnal variations of selected water quality parameters in the Arroyo Colorado and in selected sampling stations on the farms' sites. Table 2 provides a summary of the data from a study conducted during the preharvest season Analyses of the grab samples collected over a 20 h period showed a large diurnal fluctuation for each sampling station with respect to TSS, ammonia, nitrite, nitrate, total phosphorus, reactive phosphate and $CBOD_5$ levels. This high variability suggests that flow-weighted composite samples are essential for evaluating the environmental impact of the shrimp farms' effluent

on receiving waters. Since relatively high TSS levels were found in the farms' effluent water, river water samples were collected from different locations along the river to study the effect of this effluent water on the river. Samples were analyzed from 400 m (0.25 mile) upstream from the farms' discharge point, from the point of discharge and from several stations downstream from the farms' discharge point. Samples were collected during the preharvest, harvest and postharvest seasons.

THE PREHARVEST SEASON

Samples were collected at the mid-river point from water depths of 0.3 m, 0.9 m and 1.5 m. The data collected are summarized in Table 3. The TSS levels in the upstream station varied between 11 and 83 mg/L. TSS levels in the 1.5 m samples were always higher than in the samples from 0.3 m and 0.9 m water depths. The TSS levels in the river at the farms' discharge station were generally higher than those found in the upstream station. TSS levels of the river's surface water at the farms' discharge station were higher than the levels found in samples from the 0.9 m and 1.5 m water depths. The discharge surface water TSS levels varied between 23 and 220 mg/L. The data collected so far cannot explain the high fluctuation in TSS for the surface water samples of the upstream and the discharge stations. Further studies are needed to explain this variability. The TSS levels in the downstream stations show a decrease compared with the farms' discharge station. The TSS level at the 400 m (0.25 mile) downstream station was usually lower than the concentration in the upstream station. This finding suggests that the increase in the river TSS level at the farms' discharge point resulted in a TSS increase near the discharge station, with no apparent effect beyond the 400 m (0.25 mile) downstream boundary.

THE HARVEST SEASON

The harvest season samples were taken from 1.5 m, 3 m and 4.5 m. Consequently, only samples collected from the 1.5 m water depth could be compared with the corresponding values from the preharvest season. TSS levels in two out of three samples taken from the 1.5 m water depth at the discharge station were higher than the corresponding values from the preharvest season (Table 4). On the other hand in two out of three samples taken from the upstream station, the TSS level was lower than the corresponding samples from the preharvest season. This finding may suggest that the river's TSS levels at the upstream station were not affected by the farms' effluent discharges during the harvest season.

THE POSTHARVEST SEASON

Table 5 summarizes the TSS levels in the river during the postharvest season. These data provide background information regarding the river's water condition during limited effluent discharge by the farms. On October 31, 1994, there was no water discharged from TSVA into the river since all ponds were empty. Discharge from SSIon that date was less than 15,000 M^3 (4 million gallons) a day. The low levels of TSS (7-13 mg/L) recorded in all of the river sampling stations suggest that the effect of the low discharge rate from the farm could not be detected in the river. On the other hand, heavy rain on November1, 1994, resulted in a large storm water discharge into the river. It was estimated that during that 24 h period, between 190,000 and 380,000 m^3 (50 and 100 million gallons) of storm water were discharged into the river through the county drainage ditch.Consequently, elevated levels of TSS (as high as 790 mg/L) were recorded at the discharge station. This level was almost six times higher than the TSS level found during the farms' normal operation period. Elevated TSS levels were also found in the up stream sampling station (13 vs. 38 mg/L). Although high TSS levels were found at the discharge station, TSS levels at the 400 m (0.25 mile) downstream station dropped back to the normal level. This sharp decrease in TSS suggests that the increase in the river's TSS level was noticed only in the area near the discharge station. Data from samples collected on October 2 and 3, 1994, suggest that within one day of the heavy rain event, the river's TSS levels went back to normal. Water samples were also analyzed for nitrite, nitrate, total phosphorus, reactive phosphorus and pH. This monitoring was done to provide better understanding of the changes in these parameter concentrations before, during and after heavy storm water release. Table 6 summarizes the data collected on October 31, 1994, during the postharvest season when there was no discharge of storm water into the river. The data suggest that during the low shrimp farms' effluent discharge, the river nitrite level varied between 0.07 and 0.15 mg/L. These nitrite levels were in most cases higher than the October daily average value for the SSI and TSVA incoming water (0.08 mg/L). A similar trend was noticed for the river nitrate levels. The levels of total phosphorus, reactive phosphorus and pH were similar to the October daily averages in the incoming water for both farms (Tables 14 and 18). The data collected from the river during heavy storm water release and low farms' effluent discharge are summarized in Table 7. Elevated levels of nitrite and total phosphorus were found at the discharge station (up to 0.2 and 0.65 mg/L for NO, and TP, respectively), compared with the previous day's levels. On the other hand, the changes in nitrate and reactive phosphorus levels for the same period were small.

Tables 8 and 9 summarize the data from samples taken in the first and second day after the rain event, respectively. On the first day, the levels of total phosphorus in the upstream, the discharge and the 400 m (0.25 mile) downstream stations were higher than normal. These lev-

Table 4. Changes in total suspended solids (TSS) levels in different stations along the Arroyo Colorado **during the 1994 harvest season.**

	TSS[a] (mg/L)											
Date	400 m upstream			Discharge			800 m downstream			1600 m		
	0.3[a]	3.0	4.5	0.3	3.0	4.5	03	3.0	4.5	0.3	3.0	4.5
9/15/94	13	15	11	58	67	49	29	52	16	23	47	16
9/25/94	21	27	19	61	71	54	33	57	18	29	51	19
10/2/94	15	21	19	72	83	47	37	64	29	33	57	27

[a]TSS

[b]Water depth (in meters) from which samples were taken

Table 5. Total suspended solids (TSS) levels in different stations along the Arroyo Colorado during low farms' effluent discharges and different levels of storm water releases at the 1994 postharvest season.

	TSS[a] (mg/L)								
Date	400 mupstream			Discharge			400 m downstream		
	0.3a	1.5	3.0	0.3	1.5	3.0	0 3	1.5	3.0
10/31	13	13	12	13	13	7	N/A	N/A	N/A
11/1	34	3 8	N/A	610	790	405	18	19	N/A
11/2	7	8	N/A	N/A	11	11	N/A	48	74
11/3	N/A	7	2	N/A	9	12	10	13	N/A
	800 m downstream			1200 m downstream			1600 m downstream		
	0.3[a]	1.5	3.0	0 3	1.5	3.0	0 3	1.5	3.0
10/31	15	12	12	N/A	N/A	N/A	12	12	14
11/1	20	17	23	20	21	10	19	19	15
11/2	N/A	47	69	N/A	24	21	N/A	22	19
11/3	13	9	N/A	14	12	N/A	N/A	N/A	N/A
	2400 m downstream			3200 m downstream			4800 m downstream	6400 m downstream	8000 m downstream
	0.3[b]	1.5	3.0	0.3	1.5	3.0	0.3	1.5	3.0
10/31	9	10	12	10	10	9	N/A	N/A	N/A
11/1	N/A	N/A	N/A	N/A	20	N/A	20	20	20
11/2	N/A	N/A	N/A	N/A	10	N/A	23	8	25
11/3	N/A	N/A	N/A	N/A	8	N/A	20	14	15

[a]TSS

[b]Water depth (in meters) from which samples were taken

els returned to normal only on the second day after the heavy rain event. On the other hand, the heavy rain did not result in a significant change in reactive phosphorus levels in most of the sampling stations on the river. Nitrite at the discharge station was still higher than normal on the first day after the heavy rain. On the second day after the rain event, the nitrite levels in the upstream and discharge stations dropped to the level found before the rain. A steady decrease was noticed in the nitrate levels in most stations during the two days following the heavy rain. These data

Table 6. Changes in selected water quality parameters along the Arroyo Colorado during low farms' effluent discharges and no storm water releases.

Sampling station	NO_2-N (mg/L)	NO_3-N (mg/L)	TP[a] (mg/L)	RP[b] (mg/L)	pH
400 m upstream 0.3 m[c]	0.12	1.00	0.32	0.24	8.3
400 m upstream 1.5 m	0.13	1.10	0.33	0.27	8.3
400 m upstream 3.0 m	0.13	0.70	0.28	0.19	8.2
Discharge 0.3 m	0.12	0.90	0.35	0.27	8.3
Discharge 1.5 m	0.13	1.10	0.34	0.27	8.2
Discharge 3.0 m	0.15	0.70	0.35	0.25	8.1
800 m downstream 0.3 m	0.13	1.00	0.35	0.26	8.4
800 m downstream 1.5 m	0.14	1.20	0.35	0.26	8.3
800 m downstream 3.0 m	0.13	0.80	0.37	0.25	8.2
1600 m downstream 0.3 m	0.12	1.10	0.36	0.28	8.3
1600 m downstream 1.5 m	0.13	1.10	0.38	0.27	8.3
1600 m downstream 3.0 m	0.09	0.60	0.26	0.19	8.2
2400 m downstream 0.3 m	0.13	1.10	N/A	0.25	8.2
2400 m downstream 1.5 m	0.13	1.00	N/A	0.29	8.3
2400 m downstream 3.0 m	0.09	0.70	N/A	0.18	8.3
3200 m downstream 0.3 m	0.13	0.90	N/A	0.29	8.2
3200 m downstream 1.5 m	0.13	0.90	N/A	0.29	8.2
3200 m downstream 3.0 m	0.07	0.70	N/A	0.15	8.3

[a] Total phosphorus
[b] Reactive phosphorus
[c] Water depth (in meters) from which samples were taken

Table 7. Changes in selected water quality parameters along the Arroyo Colorado during the farms' low effluent discharges and heavy storm water releases.

Sampling station	NO_2-N (mg/L)	NO_3-N (mg/L)	TP[a] (mg/L)	RP[b] (mg/L)
400 m upstream 0.3 m[c]	0.13	1	0.31	0.23
400 m upstream 1.5 m	0.14	1.2	0.31	0.25
Discharge 0.3 m	0.2	1	0.59	0.2
Discharge 1.5 m	0.14	0.8	0.65	0.17
Discharge 3.0 m	0.12	0.7	0.42	0.19
400 m downstream 0.3 m	0.11	1.1	0.33	0.25
400 m downstream 1.5 m	0.13	1.2	0.37	0.25
800 m downstream 0.3 m	0.12	1	0.32	0.25
800 m downstream 1.5 m	0.12	1.1	0.37	0.25
800 m downstream 3.0 m	0.06	0.6	0.24	0.12
1200 m downstream 0.3 m	0.11	1	0.32	0.24
1200 m downstream 1.5 m	0.11	1	0.33	0.25
1200 m downstream 3.0 m	0.04	0.6	0.19	0.06
1600 m downstream 0.3 m	0.17	1	N/A	N/A
1600 m downstream 1.5 m	0.12	1	0.37	0.25
1600 m downstream 3.0 m	0.05	0.6	0.18	0.08
3200 m downstream 1.5 m	0.12	0.9	0.37	0.24
4800 m downstream 1.5 m	0.12	0.9	0.34	0.24
6400 m downstream 1.5 m	0.12	0.8	0.34	0.22
8000 m downstream 1.5 m	0.02	0.7	0.22	0.1

[a] Total phosphorus
[b] Reactive phosphorus
[c] Water depth (in meters) from which samples were taken

Table 8. Changes in selected water quality parameters along the Arroyo Colorado, one day after heavy rain during farms' low effluent discharges and no storm water releases.

Sampling station	NO_2-N (mg/L)	NO_3-N (mg/L)	TP[a] (mg/L)	RP[b] (mg/L)
400 m upstream 0.3 m[c]	0.12	0.7	0.31	0.24
400 m upstream 1.5 m	0.12	0.7	0.4	0.24
Discharge 0.3 m	0.13	0.7	0.34	0.22
Discharge 1.5 m	0.14	0.6	0.36	0.25
Discharge 3.0 m	0.19	0.4	0.27	0.25
400 m downstream 0.3 m	0.24	0.8	N/A	N/A
400 m downstream 1.5 m	0.25	0.9	0.4 1	0.19
400 m downstream 3.0 m	0.23	0.8	0.42	0.22
800 m downstream 0.3 m	0.18	0.8	0.29	0.27
800 m downstream 1.5 m	0.12	0.4	0.36	0.24
800 m downstream 3.0 m	0.18	0.6	0.23	0.18
1200 m downstream 0.3 m	0.17	0.7	0.32	0.24
1200 m downstream 1.5 m	0.14	0.7	0.26	0.24
1200 m downstream 3.0 m	0.03	0.3	0.21	0.14
1600 m downstream 0.3 m	0.07	0.6	0.31	0.24
1600m downstream 1.5 m	0.08	0.5	0.32	0.24
1600 m downstream 3.0 m	0.04	0.5	0.25	0.14
3200 m downstream 1.5 m	0.11	0.6	0.32	0.22
4800 m downstream 1.5 m	0.06	0.5	0.36	0.16
6400 m downstream 1.5 m	0.07	0.8	0.27	0.16
8000 m downstream 1.5 m	0.09	1	0.29	0.2

[a] Total phosphorus
[b] Reactive phosphorus
[c] Water depth (in meters) from which samples were taken

Table 9. Changes in selected water quality parameters along the Arroyo Colorado, two days after heavy rain during low farms' effluent discharges and no storm water releases.

Sampling station	NO_2-N (mg/L)	NO_3-N (mg/L)	TP[a] (mg/L)	RP[b] (mg/L)
400 m upstream 0.3 m[c]	0.08	0.5	0.29	0.18
400 m upstream 1.5 m	0.08	0.5	0.29	0.21
400 m upstream 3.0 m	0.08	0.4	0.29	0.21
Discharge 0.3 m	0.09	0.5	0.32	0.21
Discharge 1.5 m	0.09	0.5	0.31	0.21
Discharge 3.0 m	0.06	0.3	0.33	0.15
400 m downstream 0.3 m	0.09	0.5	0.29	0.22
400 m downstream 1.5 m	0.1	0.5	0.32	0.21
800 m downstream 0.3 m	0.1	0.6	0.28	0.23
800 m downstream 1.5 m	0.1	0.6	0.26	0.21
1200 m downstream 0.3 m	0.1	0.5	0.29	0.22
1200 m downstream 1.5 m	0.1	0.5	0.3	0.23
3200 m downstream 1.5 m	0.11	0.5	0.29	0.19
4800 m downstream 1.5 m	0.1	0.5	0.27	0.17
6400 m downstream 1.5 m	0.1	0.5	0.23	0.17
8000 m downstream 1.5 m	0.1	0.5	0.27	0.2
7200 m surface	0.11	0.5	0.28	0.2
7200m; 1.5 m	0.11	0.5	0.28	0.2

[a] Total phosphorus
[b] Reactive phosphorus
[c] Water depth (in meters) from which samples were taken

suggest that during heavy storm water release, a significant increase in total phosphorus, nitrite and nitrate can be expected by the discharge station. The effect was mostly localized, with no significant effect on the river's water at a distance of 400 m (0.25 mile) below the discharge point. Further studies are needed to explain the decrease in nitrate levels in the river water in the two days following the heavy storm water release.

INFLUENT AND EFFLUENT CHARACTERIZATION OF THREE AQUACULTURE FACILITIES IN SOUTH TEXAS

The following is a summary of the study conducted at two commercial shrimp farms, Taiwan Shrimp Village Association (TSVA) and Harlingen Shrimp Farm (HSF), and one eel farm, Southern Star Inc. (SSI). These three farms are located in south Texas along the Gulf of Mexico. Two of the farms, TSVA and SSI, pump their water from a small river, the Arroyo Colorado, which also receives the farms' effluent. The other farm, HSF, pumps its water from the Laguna Madre, a shallow hypersaline lagoon which also receives the farm's effluent water. All three farms discharge their effluent waters into existing county drainage ditches, originally designed to drain agricultural runoff and storm waters. During the 1994 production season, only 79 of the 85 2-ha ponds of TSVA were stocked with postlarvae (PL) of *P. vannamei*. The average stocking density of the farm was 50 PL/m2, with an average yield of about 4,600 kg/ha. Each pond was equipped with 10 to 12 two-horsepower paddlewheel aerators. The farm's average daily water exchange was 10%, with an average FCR value of more than 2. The SSI farm has 95 2 ha growout ponds and 28 nursery ponds that varied in size between 200 and 500 m^2. During the 1994 production season, only 15.6 of the farm's 193 ha were stocked with American eels (*Anguilla rostrate*). Only one 2 ha pond on the farm was stocked with shrimp (*P. vannamei*) at low density (19 PL/m^2). The farm's annual production was 33,000 kg of eels and about 5,200 kg of shrimp. The HSF has a total of 34 growout ponds that varied in size between 0.023 and 2.02 ha Only 150 out of the 183 ha available an the farm were stocked with *P. vannamei* (15 PL/m^2) during the 1994 season. The farm's average yield was 1,800 kg/ha, with 7% daily water exchange and an FCR value of 2.7.

CHARACTERIZATION OF INTAKE AND EFFLUENT WATERS OF TAIWAN SHRIMP VILLAGE ASSOCIATION (TWA) FARM

Water analyses were made on samples taken from two main discharge gates: outlet TV3 which drained water from pond #1 through pond #25 and outlet TV2 which received effluent water from pond #26 through pond #68. Ponds #69 through #85 were drained directly into the county drainage canal (CDC). Since this ditch was fed also by effluent from the SSI farm and by agricultural runoff and/or storm waters, the effluent water from these ponds was not monitored. Table 10 summarizes the changes in selected water quality parameters in five sampling stations on and near the farm.

Total Suspended Solids (TSS)

The level of TSS in the incoming water Of this farm varied between 0 and 3.5 mg/L (Table 10), with a daily average of 13.4 mg/L for the whole sampling period. The daily averages by month suggest that TSS values of the incoming water stayed relatively steady throughout the growing season. This finding may suggest that the combined discharges from TSVA, SSI and CDC did not affect the TSS level of the Arroyo Colorado at the intake station of TSVA farm. From July through September, the daily average levels of TSS in the effluent water from stations TV2 and TV3 varied between 80.4 and 124.0 mg/L. As the number of ponds in production during October decreased due to harvest, so did the monthly average of TSS levels of these outlets (45.2 and 74.6 mg/L for TV2 and TV3, respectively). The TSS monthly average for August and October in TV3 was higher than the corresponding levels from TV2. The reason for this higher TSS level is not clear; further studies are needed to explain these differences. The seasonal daily average of TSS levels for the main discharge canal (MDC) was similar to the values recorded for TV2 and TV3. Except for the high TSS monthly average in September (306 mg/L) in the CDC, the monthly averages for the other months were mostly in the 50 mg/L range. Quantification of CDC contribution is needed to fully assess the effect of this source on the river. The effluent TSS level in this farm was much lower than the daily average (183 mg/L) reported by Hopkins and coworkers (1993) for effluent from South Carolina shrimp ponds that were stocked at 44 PL/m^2.

To better understand the changes in TSS and VSS levels during harvest, effluent samples were collected from the outlets of three ponds on the farm. Table 11 provides a summary of the data collected from one of the ponds. As a reference point, readings were also taken from the TV1 (farm's intake), TV2 (combined drain outlet for ponds #26 through #68), TV3 (combined dram outlet for ponds #1 through #25) and MDC (combined outlet for CDC, TSVA and SSI farms). During the three day period, the TSS level at the outlet of pond #24 varied between 41 and 945 mg/L, with the highest reading found in the last sample. The VSS levels for this period varied between 15 and 786 mg/L, with the highest value observed again in the last sample. These findings suggest that as the water level in the pond decreased, a larger amount of organic matter began to appear. For the same sampling period, TSS levels in TV3 varied between 59 and 68 mg/L. The data suggest that the VSS portions of the TSS at the TV3 station were often

Table 10. Changes in total suspended solids, total phosphorus, and reactive phosphorus in different sampling stations on Taiwan Shrimp Village Association (TSVA) farm during the 1994 production season.

Period	value	TSS[a] (mg/L)					Total P[g] (mg/L)					Reactive P[h] (mg/L)			
		TV1[b]	TV2[c]	TV3[d]	MDC[e]	CDC[f]	TV1	TV2	TV3	MDC	CDC	TV1	TV2	TV3	MDC
Jul.	Av.[i]	13	107	103	124	50	NA	NA	NA	NA	NA	0.09	0.22	0.18	0.22
Aug.	Av.	16	80	108	92	50	0.29	0.55	0.47	0.53	0.32	NA	NA	NA	NA
Sep.	Av.	13	124	104	108	306	0.27	0.51	0.43	0.52	0.22	0.14	0.38	0.27	0.31
Oct.	Av.	10	45	75	76	50	0.31	0.47	0.46	0.46	0.18	0.20	0.33	0.29	0.33
Jul.-	Av.	13	93	99	101	79	0.29	0.51	0.45	0.50	0.28	0.12	0.27	0.22	0.28
Oct.	STD[j]	7	47	37	36	87	0.05	0.08	0.07	0.08	0.10	0.06	0.10	0.08	0.07
	Max	35	220	235	203	306	0.38	0.65	0.56	0.66	0.45	0.21	0.45	0.32	0.37
	Min	0	12	38	58	30	0.21	0.38	0.32	0.39	0.18	0.04	0.11	0.04	0.15
	n[k]	35	35	35	19	10	14	14	14	13	6	10	10	10	7

[a] Total suspended solids
[b] **Sampling station at the water intake** of TSVA farm
[c] Sampling station at the discharge gate for pond #26 through pond #68 of TSVA farm
[d] Sampling station at the discharge gate for pond #1 through pond #25 of TSVA farm
[e] Sampling station at the discharge outlet for county discharge canal (CDC), TSVA, and Southern Star Inc. farms
[f] Sampling station at the CDC
[g] Total phosphorus
[h] Reactive phosphorus
[i] Average
[j] Standard deviation
[k] Number of observations

Table 11. TSS and VSS levels in one pond outlet and in selected sampling stations on TSVA farm during harvest.

Date	Time	Pond #24 TSS[a] (mg/L)	Pond #24 VSS[b] (%)	TV1[c] TSS (mg/L)	TV3[d] TSS (mg/L)	TV3[d] vss (%)	MDC[e] TSS (mg/L)	MDC[e] vss (%)
9/16/94	1:00 pm	41	37	21	61	23	57	33
9/16/94	7:00 pm	69	32		60	20	58	40
9/17/94	1:00 am	91	23		59	10	58	21
9/17/94	7:00 am	121	16		59	10	59	19
9/17/94	1:00 pm	159	26		61	25	61	20
9/17/94	7:00 pm	251	45		64	39	62	34
9/18/94	1:00 am	315	49		65	38	63	46
9/18/94	7:00 am	394	28		64	48	63	38
9/18/94	1:00 pm	560	38		66	33	65	54
9/18/94	7:00 pm	652	49		66	36	66	47

[a] Total suspended solids
[b] Volatile suspended solids
[c] Sampling station at the water intake of Taiwan Shrimp Village Association (TSVA) farm
[d] Sampling station at the discharge gate for pond #1 through pond #25 of TSVA farm
[e] Sampling station at the discharge outlet for county discharge canal (CDC), TSVA, and Southern Star Inc. farms

Table 12. Comparison of TSS levels in ponds' effluent water from TSVA farm and Harlingen Shrimp farm during the 1994 production season.

	Pond 60 Taiwan Shrimp Village Association Farm							
Date	8/11	8/18	8/25	9/01	9/08	9/15	9/22	9/29
Av[a] TSS[b] (mg/L) 79.5	74	79	93	50	138	13	7	182
	Pond G9 Harlingen Shrimp Farm							
Date	7-20	7-27	8-03	8-10	8-17	8-24	9-07	9-14
Av. TSS (mg/L) 64.4	111	30	42	42	109	89	54	38

[a] Average
[b] Total suspended solids

lower than the corresponding values for samples taken at pond #24's outlet; The TSS levels at the MDC outlet were similar to the levels recorded at the TV3 outlet. However, a higher VSS proportion was found in the MDC samples, Since MDC received water from different sources, it is not possible to single out the primary source for the higher VSS portion. Furthermore, the TSS levels at TV3 suggest that although the pond effluent water had high levels of TSS (up to 945 mg/L), this level was reduced by 93% at the TV3 outlet. Even if we are to ignore the particle load contributions from the other 24 ponds draining into TV3 outlet it is obvious that the drainage canal served as a settling basin for the pond's effluent water. In terms of VSS, the data suggest that by the time the water from this pond reached the TV3 outlet, only 46% of the TSS was in the form of VSS compared to 83% at the pond's outlet site. Table 12 provides a summary of the TSS values found in samples taken from pond #60 outlet in TSVA farm and pond G9 outlet in HSF during the 1994 production season. The levels of TSS for the TSVA pond varied between 7 and 182 mg/L, with a daily average of 79.5 mg/L. The TSS values for the HSF pond G9 varied between 30 and 11 I mg/L, with a daily seasonal average of 64.4 mg/L. Stocking density in pond #60 was 50 PL/m^2, while in the other pond it was only 13 PL/m^2. The data suggest that lower stocking density does not necessarily result in lower levels of TSS in the effluent water.

Total Phosphorus (TP)

The monthly average level of TP in the incoming water (TV1 station) varied between 0.21 and 0.38 mg/L, with no increase from the early growing to the harvest season (Table 10). The daily average TP for the whole season was 0.29 mg/L. The seasonal average level of TP in outlets TV2 and TV3 varied between 0.32 and 0.65 mg/L, with a daily average of 0.48 mg/L. Higher seasonal TP average value (0.51 vs. 0.45 mg/L) was observed for TV2 station as this outlet drained twice as many ponds as the other. The monthly averages of TP in the farm's effluent waters did not show an increase over time. A slight decrease in the monthly average TP was noticed in samples from TV2 from the early season to harvest. The TP seasonal average for MDC was just a little higher than the combined average for TV2 and TV3 stations (0.5 vs. 0.48 mg/L). Although this outlet received water from other sources (e.g., SSI farm and CDC), the TP level in this station was not greatly affected. Assuming a daily usage of 379,000 M^3 (100 million gallons) at peak pumping requirement with a net increase of 0.18 mg/L TP over the inffluent water, then the TP releases by the farm could be about 72 kg/day or 165 kg (363 lb) of P_2O_5/day. This quantity of P_2O_5 is about half the regular phosphorus application for 1 ha cropland (180 kg/ha) applied at least twice in each cycle.

Reactive Phosphorus (RP)

The monthly average level of RP in the incoming water varied between 0.09 and 0.20 mg/L, with a seasonal daily average of 0.12 mg/L (0.04 to 0.20 mg/L range). A slight increase in RP was noticed from July to October (Table 10). The seasonal averages of RP for TV2 and TV3 stations were 0.27 and 0.22 mg/L, respectively, with a 0.04 to 0.45 mg/L range. The combined seasonal daily average for the two stations was overtwice the level recorded for the intake (0.12 vs. 0.25 mg/L). The seasonal average of RP for the MDC station was a little higher than the corresponding average of TV2 and TV3 (0.28 vs. 0.25 mg/L). Here again, a slight increase in RP from the early season to harvest was noticed.

Table 13. Changes in pH, dissolved oxygen, and five-day carbonaceous oxygen demand in different sampling stations on Taiwan Shrimp Village Association (TSVA) farm during the 1994 production season.

Period	value	pH				DO[f] (mg/L)				$CBOD_5$[g] (mg/L)				
		TV1[a]	TV2[b]	TV3[c]	MDC[d]	TV1	TV2	TV3	MDC	TV1	TV2	TV3	MDC	CDC[e]
Jul.	Av.[h]	8.5	7.9	7.9	7.8	7.2	5.6	5.5	6.0	4.1	3.7	3.7	3.5	5.1
Aug.	Av.	8.4	7.7	7.8	7.7	6.8	4.8	5.7	5.7	3.8	3.4	3.8	3.2	3.4
Sep.	Av.	8.4	7.6	7.8	7.7	6.6	4.2	5.1	5.0	4.5	2.4	2.8	2.6	15.0
Oct.	Av.	8.3	7.9	7.8	7.9	5.7	4.7	3.4	4.6	2.4	2.5	4.1	3.0	NA
Jul.-.	Av.	8.4	7.8	7.8	7.8	6.8	4.7	5.3	5.5	3.8	2.9	3.6	3.1	5.5
Oct	STD[i]	0.2	0.2	0.1	0.1	0.9	0.9	1.0	1.0	1.8	1.0	1.5	1.0	4.5
	Max	8.7	8.2	8.1	8.0	10.2	6.8	7.2	8.8	11	4.8	8.4	5.0	15.0
	Min	8.0	7.5	7.7	7.6	4.8	2.8	2.6	1.0	1.3	1.4	1.1	1.7	1.6
	n[j]	31	31	31	18	84	68	68	79	32	31	32	19	7

[a] Sampling station at the water intake of TSVA farm
[b] Sampling station at the discharge gate for pond #26 through pond #68 of TSVA farm
[c] Sampling station at the discharge gate for pond #1 through pond #25 of TSVA farm
[d] Sampling station at the discharge outlet for county discharge canal (CDC), TSVA, and Southern Star Inc. farms
[e] Sampling station at the CDC
[f] Dissoived oxygen
[g] Five-day carbonaceous biochemical oxygen demand
[h] Average
[i] Standard deviation
[j] Number of observations

pH

The seasonal average pH level in the incoming water was 8.4, with an 8.0 to 8.7 range (Table 13). The seasonal averages of pH levels in stations TV2 and TV3 were lower than the pH of the incoming water (7.8 vs. 8.4). These values were well within the daily average range (6.0 to 9.0) set by the TNRCC.

Dissolved Oxygen (DO)

The seasonal average of DO in the farm's incoming water was 6.8, with a 4.8 and 10.2 mg/L range (Table 13). A slight decrease in DO level in the river's water was observed from the early season to the harvest. The seasonal average of the DO level for TV3 station was a little higher than the corresponding value for TV2 (5.3 vs. 4.7, with a 2.6 to 7.2 mg/L range). The seasonal average of DO concentration for station MDC was a little higher than the combined value for the TV2 and TV3 stations (5.6 vs. 5.0 mg/L). The monthly and seasonal averages of DO concentration for both the TV2 and TV3 stations were lower than the 6.0 mg/L daily average required by the permit. An increase **in effluent DO concentration will be needed** to meet regulatory requirements.

Five-day Carbonaceous Biochemical Oxygen Demand ($CBOD_5$)

The seasonal daily average of CBOD, in the incoming water was 3.8 mg/L, with a 1.3 and 10.9 mg/L range (Table 13). The seasonal averages of $CBOD_5$ in samples from TV2 and TV3 were 2.9 and 3.6 mg/L, respectively, with a 1.1 and 8.4 mg/L range. The seasonal average for the two outlets was 3.3 mg/L. The data collected so far do not explain why a higher level of CBOD, was found in TV3 compared with TV2. The seasonal average value for the MDC station was a little lower than the combined value for TV2 and TV3 (3.1 vs. 3.3 mg/L). The seasonal average of $CBOD_5$ value for the CDC station was the highest among all stations (5.5 mg/L). The higher $CBOD_5$ level in the incoming water, compared with the reading from TV2, TV3 and MDC outlets, suggests that circulating the river water in the farm's ponds reduces the level of $CBOD_5$. Furthermore, the data suggest that the river's $CBOD_5$ was controlled by factors other than the farm's effluent water. Since the seasonal daily average of $CBOD_5$ level of the farm's effluent water was lower than the corresponding values for the incoming **water, meeting the standard will not** create any problem. All of the $CBOD_5$ levels found in this farm were **lower than the** 8.5 mg/L BOD level reported

Table 13. **Changes in pH, dissolved oxygen, and five-day carbonaceous oxygen demand in different sampling stations on Taiwan Shrimp Village Association (TSVA) farm during the 1994 production season.**

Period	value	pH				DO[f] (mg/L)				$CBOD_5$[g] (mg/L)				
		TV1[a]	TV2[b]	TV3[c]	MDC[d]	TV1	TV2	TV3	MDC	TV1	TV2	TV3	MDC	CDC[e]
Jul.	Av.[h]	**8.5**	**7.9**	**7.9**	**7.8**	**7.2**	**5.6**	**5.5**	**6.0**	**4.1**	**3.7**	**3.7**	**3.5**	**5.1**
Aug.	**Av.**	**8.4**	**7.7**	**7.8**	**7.7**	**6.8**	**4.8**	**5.7**	**5.7**	**3.8**	**3.4**	**3.8**	**3.2**	**3.4**
Sep.	Av.	**8.4**	**7.6**	**7.8**	**7.7**	**6.6**	**4.2**	5.1	5.0	**4.5**	**2.4**	**2.8**	**2.6**	15.0
Oct.	Av.	**8.3**	**7.9**	**7.8**	**7.9**	**5.7**	**4.7**	**3.4**	**4.6**	**2.4**	**2.5**	**4.1**	**3.0**	**NA**
Jul.-.	Av.	8.4	7.8	7.8	7.8	6.8	4.7	5.3	5.5	3.8	2.9	3.6	3.1	5.5
Oct	STD[i]	0.2	0.2	0.1	0.1	0.9	0.9	1.0	1.0	1.8	1.0	1.5	1.0	4.5
	Max	8.7	8.2	8.1	8.0	10.2	6.8	7.2	8.8	11	4.8	8.4	5.0	15.0
	Min	8.0	7.5	7.7	7.6	4.8	2.8	2.6	1.0	1.3	1.4	1.1	1.7	1.6
	n[j]	31	31	31	18	84	68	68	79	32	31	32	19	7

[a] Sampling station at the water intake of TSVA farm
[b] Sampling station at the discharge gate for pond #26 through pond #68 of TSVA farm
[c] Sampling station at the discharge gate for pond #1 through pond #25 of TSVA farm
[d] Sampling station at the discharge outlet for county discharge canal (CDC), TSVA, and Southern Star Inc. farms
[e] Sampling station at the CDC
[f] Dissoived oxygen
[g] Five-day carbonaceous biochemical oxygen demand
[h] Average
[i] Standard deviation
[j] Number of observations

pH

The seasonal average pH level in the incoming water was 8.4, with an 8.0 to 8.7 range (Table 13). The seasonal averages of pH levels in stations TV2 and TV3 were lower than the pH of the incoming water (7.8 vs. 8.4). These values were well within the daily average range (6.0 to 9.0) set by the TNRCC.

Dissolved Oxygen (DO)

The seasonal average of DO in the farm's incoming water was 6.8, with a4.8 and 10.2 mg/L range (Table 13). A slight decrease in DO level in the river's water was observed from the early season to the harvest. The seasonal average of the DO level for TV3 station was a little higher than the corresponding value for TV2 (5.3 vs. 4.7, with a 2.6 to 7.2 mg/L range). The seasonal average of DO concentration for station MDC was a little higher than the combined value for the TV2 and TV3 stations (5.6 vs. 5.0 mg/L). The monthly and seasonal averages of DO concentration for both the TV2 and TV3 stations were lower than the 6.0 mg/L daily average required by the permit. An increase **in effluent DO concentration will be needed** to meet regulatory requirements.

Five-day Carbonaceous Biochemical Oxygen Demand ($CBOD_5$)

The seasonal daily average of CBOD, in the incoming water was 3.8 mg/L, with a 1.3 and 10.9 mg/L range (Table 13). The seasonal averages of $CBOD_5$ in samples from TV2 and TV3 were 2.9 and 3.6 mg/L, respectively, with a 1.1 and 8.4 mg/L range. The seasonal average for the two outlets was 3.3 mg/L. The data collected so far do not explain why a higher level of CBOD, was found in TV3 compared with TV2. The seasonal average value for the MDC station was a little lower than the combined value for TV2 and TV3 (3.1 vs. 3.3 mg/L). The seasonal average of $CBOD_5$ value for the CDC station was the highest among all stations (5.5 mg/L). The higher $CBOD_5$ level in the incoming water, compared with the reading from TV2, TV3 and MDC outlets, suggests that circulating the river water in the farm's ponds reduces the level of $CBOD_5$. Furthermore, the data suggest that the river's $CBOD_5$ was controlled by factors other than the farm's effluent water. Since the seasonal daily average of $CBOD_5$ level of the farm's effluent water was lower than the corresponding values for the incoming **water, meeting the standard will not** create any problem. All of the $CBOD_5$ levels found in this farm were **lower than the** 8.5 mg/L BOD level reported

Table 14. Changes in ammonia, nitrite, and nitrate in different sampling stations at Taiwan Shrimp Village Association (TSVA) farm during the 1994 production season.

Period	value	NH_3-N (mg/L) TV1[a]	TV2[b]	TV3[c]	MDC[d]	CDC[e]	NO_2-N (mg/L) TV1	TV2	TV3	MDC	CDC	NO_3-N (mg/L) TV1	TV2	TV3	MDC	CDC
Jul.	Av.[f]	0.07	1.22	1.10	1.2	0.0	0.35	0.28	0.28	0.21	0.12	NA	NA	NA	NA	NA
					1.7	0.4										
		0.30	1.82	1.72			0.06	0.47	0.29	0.41	0.34	0.26	0.58	0.46	0.64	0.40
Aug.	Av.				4	7										
					1.2	0.0										
		0.27	1.44	0.89			0.06	0.51	0.20	0.43	0.05	0.35	0.80	0.45	0.83	0.80
Sep.	Av.				1	7										
					0.7	0.0										
		0.26	1.00	0.75			0.10	0.33	0.15	0.29	0.01	0.53	0.63	0.43	0.53	0.50
Oct.	Av.				6	3										
					1.3	0.2										
Jul.-		0.23	1.40	1.14			0.13	0.41	0.23	0.35	0.21	0.36	0.67	0.45	0.68	0.48
Oct.	Av.				5	8										
					0.4	0.4										
		0.14	0.48	0.47			0.22	0.13	0.08	0.14	0.26	0.21	0.14	0.09	0.15	0.28
	STD[g]				7	0										
					2.1	1.2										
		0.49	2.36	2.08			0.90	0.59	0.39	0.52	0.81	0.80	0.90	0.60	0.90	0.80
	Max				7	5										
					0.6	0.0										
		0.01	0.16	0.43			0.03	0.21	0.14	0.00	0.01	0.10	0.50	0.30	0.50	0.10
	Min				0	0										
	n[h]	33	33	33	20	9	15	15	15	15	8	12	12	12	11	5

[a] Sampling station at the water intake of TSVA farm
[b] Sampling station at the discharge gate for pond #26 through pond #68 of TSVA farm
[c] Sampling station at the discharge gate for pond #1 through pond #25 of TSVA farm
[d] Sampling station at the discharge outlet for county discharge canal (CDC), TSVA, and Southern Star Inc. farms
[e] Sampling swim at the CDC
[f] Average
[g] Standard deviation
[h] Number of observations

by Hopkins and co-workers (1993) for pond effluent in South Carolina.

Ammonia (NH_3-N)

The farm's seasonal average of ammonia level in the incoming water (TV1 station) was 0.23 mg/L, with a 0.01 to 0.49 mg/L range (Table 14). July's average (0.07 mg/L) was about four times lower than the averages for the following three months. Ammonia level in the intake station for July stayed low in spite of the fact that the average for this month at the MDC station was 1.29 mg/L. Furthermore, although the ammonia average for the MDC station for September and October was significantly different (1.21 and 0.76 mg/L, respectively), the monthly average at TV 1 for these months was about the same. Mom in-depth study is needed to decide whether ammonia loads at MDC can affect the level found in the farm's intake station. The seasonal ammonia average for TV2 (1.40, with a 0.16 to 2.36 mg/L range) was higher than the level at TV3 (1.14, with a 0.43 to 2.08 mg/L range). A **decreasing** trend in the ammonia monthly average was evident from August to October. This decrease is probably a result of lower discharge volume since some ponds had been harvested The seasonal average concentration of ammonia for station MDC was 1.35, with a 0.6 to 2.17 mg/L range. Monthly averages for this station were usually lower than the corresponding values from TV2. Except for one month (October), the farm's effluent monthly averages for ammonia were higher than the 1 mg/L limit set by the regulatory agency. These values were much higher than the 0.08 mg/L daily average reported by Hopkins and co-workers (1993) for shrimp ponds stocked at 44 PL/m^2 with a 25% daily

water exchange. It is possible that the low level reported from South Carolina's effluent water was a result of ammonia uptake by the algae. This conclusion is supported by the low seasonal average of ammonia level (0.03 mg/L) found in HSF effluent water, where algal concentration was extremely high. Effluent ammonia levels found on this farm were much lower than the 6.5 mg/L values reported by Chen and co-workers (1986, 1989) for intensive shrimp ponds in Taiwan. The farm's effluent ammonia levels were a little higher than the "safe level" of 1.1-1.4 mg/L reported in the literature for larvae and juvenile shrimp (Wickins 1976, Chen and Chin 1988, Chin and Chen 1988, Wajsbrot et *al.* 1990). The farm's values were often lower than the 96 h LC_{50} values (0.4-3.1 mg/L range) reported for fish (Ball 1967, Colt and Tchobanoglous 1976) and the 3.3-6.4 mg/L range reported for marine mollusc (Epifanio and Srna 1975). These values were higher than the 0.050-0.2 mg/L range and found to have a "significant growth reduction on most aquatic animals" (Colt and Amstrong 198 1).

Nitrite (NO2-N)

The nitrite monthly averages of the incoming water varied between 0.06 and 0.35 mg/L, with a seasonal average of 0.13 mg/L (Table 14). The monthly nitrite averages at the MDC station were lower than the corresponding value from TV2 and TV3. The seasonal average level of nitrite in TV2 was higher than the level at the TV3 station (0.41 mg/L vs. 0.23). The seasonal averages of nitrite for TV2 and TV3 stations were higher than the levels found in the farm's incoming water. However, these nitrite levels were lower than the 0.5 mg/L value reported by Hopkins and co-workers (1993) for effluent water from ponds stocked at 44 PL/m^2. The farm's effluent nitrite levels were much lower than the 96h LC_{50} values (8.5-15.4 mg/L) reported for shrimp (Armstrong *et* al. 1976, Wickins 1976) or the 96h LC_{50} values (532 and 756 mg/L) reported for two species of shellfish (Epifanio and Sma 1975).

Nitrate (NO,-N)

The nitrate monthly averages of the incoming water varied between 0.26 and 0.53 mg/L (Table 14), with a seasonal daily average of 0.36 mg/L (0.1-0.8 mg/L range). The seasonal average nitrate level for TV2 and TV3 stations was 0.67 and 0.45 mg/L, respectively. The monthly averages from these stations were higher than the corresponding values from the intake station. The nitrate monthly averages for August and September at the MDC station were a little higher than the corresponding values from TV2. Nitrate levels at CDC were almost as high as the values from TV2. The nitrate level reported by Hopkins and co-workers (1993) for effluent water from shrimp ponds stocked at 44 PL/m^2 was about 10 times higher than the seasonal average for TV2. Wickins (1976) reported a 48h LC_{50} value of 3,400 mg/L for juvenile shrimp. Colt and Tchobanoglous (1976) reported a 96h LC_{50} value of 1,000-2,000 mg/L for fish. Epifanio and Srna (1975) reported that the 96h value for *Crassostrea virginica* varied between 2,600 and 3,800 mg/L. Only high levels of nitrate (>90 mg/L) were reported to affect growth of aquatic animals (Wickins 1976).

TSS CONTRIBUTION BY AN EARTHEN DRAINAGE CANAL AT THE TAIWAN SHRIMP VILLAGE ASSOCIATION FARM

The study was conducted on a section of a drainage canal on TSVA farm controlled by outlet TV3. This gate received effluent waters from ponds #1 through #25. Samples were collected from a section where there was no direct effluent discharge from any ponds. A total of 20 sampling stations (M 1 -M20) were set at 15.2 m (50') apart in a section with an "L" shape. Station M20 was placed about 61 m (200') from the drain pipe of pond #1 (the first pond that discharged water into this drainage ditch) while station Ml5 was positioned following the drainage canal curve. Station Ml was about 15.2 m (50') from the TV3 outlet. Table 15 summarizes the TSS data collected from these sampling stations. For comparison, data are also provided for other key sampling locations. Samples were taken from the farm's intake station (TV1), the farm's discharge point into the river (MDC) and the TV3 station.

The TSS levels in Ml7 and Ml5 were higher or similar to readings from M20. It is most likely that the increase in TSS was due to erosion of the drainage canal soil, with some amplification at the canal's turning point near M15. In a few cases, TSS levels in M1 were reduced nearly 40% compared with Ml5 readings. This reduction in TSS suggests that the drainage canal acted as a primary settling basin for the effluent water.

Table 16 provides some information regarding the VSS portion in the TSS readings from selected sampling stations. The VSS level in these samples varied between 27 and 82%, with no clear correlation in distance of the sampling station from M20. An adequate characterization of VSS is essential for the design of any aquaculture effluent treatment facility. For example, effluent water rich with unicellular algae will require a different treatment strategy to reduce its TSS level than water loaded with shrimp feces and unconsumed feed. A series of jar tests were run by an engineering company (NRS Consulting Engineers. Harhngen, Tex.) to determine settling characteristics of the water discharged from the ponds. Based on this information, it was determined that without adding flocculating agents, the settling time was too long to be practical (Norris 1994). Significant reduction in TSS was obtained when flocculating agents were used. Cost analysis of this treatment practice suggests that it may not be cost-effective. Based on data collected in this study and the information from other studies on the farm's sites (e.g., TSS

Table 15. The effect of an earthen drainage canal on effluent TSS level.

Date	TSS[a] (mg/L)													
	TV1[b]	TV3[c]	MDC[d]	M20[e]	M17	M15	M14	M11	M8	M7	M5	M3	M2	M1
Jul. 20	55	193	227	146	172		179	169	179		192		192	193
Jul. 26	46	185	24	139	161		164		181		199		191	181
Aug. 10	48	256	84	400		478			249		261		274	294
Aug. 17	36	120	176	109		151	139	139			114		118	120
Aug. 24	38	182	163	131		178		133				109		181
Sep. 16	21	61	57	95	105	102		98				85		59
Sep. 25	18	79	109	89	87	79		84		81		81		99
Oct. 2				94	82	79		81		87		93		59

[a] Total suspended solids
[b] Sampling station at the water intake for Taiwan Village Shrimp Association (TSVA) farm
[c] Sampling station at the discharge canal for ponds #1-#25 of TSVA
[d] Sampling station at the discharge canal receiving water from two farms
[e] Sampling station in a section of the drainage canal on TSVA farm

Table 16. VSS portions in TSS samples collected at different dates in five sampling stations along the drainage canal at Taiwan Shrimp Village Association (TSVA) farm.

Date	Sampling Stations ID									
	M20[a]		M15		M11		M3		M1	
	TSS[b] (mg/L)	VSS[c] (%)	TSS (mg/L)	VSS (%)	TSS (mg/L)	VSS (%)	TSS (mg/L)	VSS (%)	TSS (mg/L)	VSS (%)
Jul 20	146								193	
Jul 26	139	54							181	77
Aug 10	400	82	478	82					294	55
Aug 17	109	59	151	66	139	68			120	58
Aug 24	131	70	178	74	133	81	109	72	181	66
Sep 16	95	34	102	37	98	43	85	26	59	27
Sep 25	89	52	79	65	84	46	81	46	99	54
Oct 2	94	59	79	65	81	38	93	55	59	66

[a] Sampling station in a section of the drainage canal on TSVA farm
[b] Total suspended solids
[c] Volatile suspended solids

Table 17. Changes in selected water quality parameters in the intake and discharge water of an eel pond in Southern Star Inc. farm during the 1994 production season.

Parameter	Period	Average (mg/L)		STD[a]		Max		Min		n[b]	
		In[c]	Out[d]	In	Out	In	Out	In	Out	In	Out
TSS[e]	August-	5.9	2.1	4.9	1.9	15	5	0	0	7	10
NH_3-N[f]	October	0.28	0.36	0.07	0.33	0.38	0.8	0.19	0.01	5	7
NO_2-N		0.06	0.14	0.03	0.2	0.1	0.54	0.02	0.01	7	9
NO_3-N		0.25	0.31	0.08	0.19	0.4	0.6	0.2	0.1	8	10
Total P[g]		0.26	0.33	0.02	0.13	0.3	0.56	0.23	0.2	9	11
$CBOD_5$[h]		2.5	2.17	0.94	0.48	4.2	2.7	1.4	1.4	6	6

[a] Standard deviation
[b] Number of observations
[c] Sampling at the pond's intake
[d] Sampling at the pond's discharge
[f] Total ammonia nitrogen
[g] Total phosphorus

contribution from soil erosion, TSS load during harvest, etc.), the consultant recommended the following modifications: (1) deepening and widening the farm's drainage canals to achieve greater reduction in effluent of TSS levels; (2) reducing the drainage canal's soil erosion, and (3) pumping the pond harvest water into empty ponds to decrease TSS loads prior to final discharge.

CHARACTERIZATION OF INTAKE AND EFFLUENT WATERS OF SOUTHERN STAR INC. (SSI) FARM

At the time that this study was conducted, SSI was the only farm with a discharge permit. The effluent water generated by this farm came mostly from eel ponds since only one growout pond was stocked with shrimp. In addition to routine monitoring of the farm's incoming and effluent waters, samples were analyzed from the intake and the discharge of one of the eel ponds. This monitoring was designed to provide better understanding of the differences in effluent water quality between a pond stocked with eels and a pond stocked with shrimp. The data collected from the eel pond is summarized in Table 17. The seasonal average of TSS in the intake water for this pond was much lower than the corresponding value from the farm's intake (5.9 vs. 14.4 mg/L). The seasonal average TSS level in the effluent water from this pond was 2.1 mg/L. This TSS level was over 30 times lower than the corresponding values from the individual ponds monitored on the TSVA and HSF facilities (Tables 14). The CBOD level in the incoming water of this pond was higher than the level found in the pond effluent. Nevertheless, this level was still a little lower than the farm's seasonal average. This finding suggests a decrease in CBOD from the farm's pumping station to the pond intake. The average CBOD reading in the effluent water from this pond was similar to the level found in the farm's effluent water. The levels of ammonia, nitrite, nitrate and TP in the pond effluent water were higher than the concentrations in the pond's incoming water. The seasonal average ammonia level in the pond intake was much higher than the farm's intake level (0.28 vs. 0.10 mg/L). This increase in ammonia at the pond's inlet suggests that an organic decomposition process took place in the farm's intake canal. The average nitrite and TP levels in the incoming and effluent water of this pond were similar to the corresponding levels for the whole farm. The nitrate level in the pond intake was much lower than the level found in the farm's incoming water (0.25 vs. 0.42 mg/L). The level in the effluent water for the whole farm and for the pond was similar.

Total Suspended Solids (TSS)

Table 18 summarizes the TSS data collected from the intake and discharge stations of the SSI farm during the 1994 production season. The TSS monthly average in the farm's incoming water varied between 12.1 and 16.7 mg/L, with a seasonal average of 14.4 mg/L. No increase trend in the TSS monthly averages was found from early season to harvest in the intake station. The season's average was about 1 mg/L higher than the corresponding value from the TSVA farm. TSS monthly averages for September and October for the farm's intake were higher than the corresponding values from TSVA farm. As the intake station for the SSI farm was located upstream of the other farm, it is clear that these higher TSS levels were not a direct result of the effluent discharge from the two farms. The farm's effluent TSS level during July varied between 25 and 260 mg/L, with a daily average of 109 mg/L. Low water discharge rate coupled with high water turbidity from soil stirring activity by fish in front of the sampling station have resulted in artificially high effluent TSS values. This artifact was corrected in early August by increasing the water depth in the drainage canal. As a result, the average TSS in the effluent for August was about half the level monitored earlier (54.1 mg/L). The seasonal average of the TSS level, excluding the biased values from July, was only 50.9 mg/L. This level was far below the corresponding values from the other two farms.

Ammonia (NH_3-N)

The seasonal daily average of ammonia level in the farm's incoming water (SS1 station) was 0.10 mg/L, with a 0.00 to 0.52 mg/L range. A steady increase in ammonia monthly averages was observed from July to October (Table 18). The seasonal average ammonia for the TSVA farm intake was more than twice the level of SS1 (0.23 mg/L). An increase in the ammonia monthly average was noticed for the TSVA farm from July to August, with no significant change from August to harvest (Table 14). The data collected so far are not sufficient to decide whether the increase in the monthly ammonia concentration is a direct result of the two farms' effluent discharge into the river. The seasonal average of ammonia concentration for the farm's effluent water was 0.36 mg/L, with a 0.01 to 1.17 mg/L range. This average was much higher than the corresponding concentration from HSF effluent waters that were algal-rich. Nevertheless, the seasonal average was about four times lower than the corresponding values of the effluent water from TSVA farm (1.4 and 1.14 mg/L for TV2 and TV3, respectively; Table 14). Ammonia level was also far below the level found in the MDC station (1.35 mg/L) which received effluent water from the CDC and the two farms. No decrease in the monthly averages of ammonia effluent was observed for this farm from the early season to harvest, as was the case for the TSVA farm. The monthly averages of ammonia in the farm's effluent water were below the maximum level allowed by the permit.

Nitrite (NO_2-N)

The monthly average of nitrite level in the incoming

Table 18. Changes in total suspended solids, total phosphorus, and reactive phosphorus in the intake and the effluent discharge station of Southern Star Inc. (SSI) farm during the 1994 production season.

Period	Value	TSS[a] (mg/L)		NH_3-N[d] (mg/L)		NO_2-N (mg/L)		NO_3-N (mg/L)		Total P[e] (mg/L)	
		SS1[b]	SS2[c]	SS1	SS2	SS1	SS2	SS1	SS2	SS1	SS2
Jul.	Av.[f]	14.7	108.7	0.05	0.25	0.08	0.05	NA	NA	NA	NA
Aug.	Av.	12.1	54.1	0.10	0.45	0.04	0.14	0.26	0.34	0.21	0.35
Sep.	Av.	16.6	39.3	0.19	0.31	0.05	0.07	0.43	0.28	0.27	0.27
Oct.	Av.	16.7	59.3	0.21	0.71	0.08	0.20	0.63	0.40	0.32	0.38
Jul.-.	Av.	14.4	70.2	0.10	0.36	0.06	0.12	0.42	0.34	0.27	0.34
Oct	STD[g]	6.0	60.4	0.10	0.26	0.03	0.08	0.20	0.10	0.09	0.07
	Max	31.0	260.0	0.52	1.17	0.11	0.25	0.70	0.50	0.42	0.42
	Min	3.0	16.0	0.00	0.01	0.01	0.02	0.20	0.20	0.01	0.22
	n[h]	47	47	46	46	17	17	13	13	14	14

[a] Total suspended solids
[b] Sampling station at the Water intake of SSI farm from Arroyo Colorado
[c] Sampling station at the water discharge station of SSI farm
[d] Total ammonia nitrogen
[e] Total phosphorus
[f] Average
a Standard deviation
[h] Number of observations

Table 19. Changes in pH, dissolved oxygen, and five-day carbonaceous biochemical oxygen demand in the intake aud the effluent discharge station of Southern Star Inc. (SSI) farm during the 1994 production season.

Period	Value	pH		DO[c] (mg/L)		$CBOD_5$[d] (mg/L)	
		SS1[a]	SS2[b]	SS1	ss2	SS1	SS2
Jul.	Av.[e]	8.6	8.2	8.7	7.0	4.5	5.8
Aug.	Av.	8.5	8.1	8.7	5.9	4.5	4.7
Sep.	Av.	8.5	8.1	9.4	5.3	4.5	3.5
Oct.	Av.	8.2	7.8	6.7	5.7	2.5	1.7
Jul.-	Av.	8.4	8.0	8.3	5.7	4.0	1.7
Oct.	STD[f]	0.2	0.2	3.1	1.1	1.6	0.4
	Max	8.8	8.6	20.1	8.3	7.7	2.7
	Min	7.8	7.3	2.3	2.7	1.2	1.3
	n[g]	95	95	296	295	42	42

[a] Sampling station at the intake of SSI farm
[b] Sampling station at the SSI effluent discharge gate
[c] Dissolved oxygen
[d] Five-day carbonaceous biochemical demand
[e] Average
[f] Standard deviation
[g] Number of observations

water varied between 0.04 and 0.08 mg/L (Table 18). No increasing trend was observed in the monthly average of nitrite levels from early season to harvest. The seasonal daily average was 0.06 mg/L. This average was considerably lower than the corresponding value from the TSVA farm (0.13 mg/L; Table 14). The data collected so far do not support nor reject the hypothesis that the effluent water discharge from SSI and TSVA affected the nitrite levels in the incoming water of the two farms. The seasonal average of nitrite level for the farm's effluent was 0.12 mg/L. No increase was observed in the monthly averages of the nitrite in the farm's effluent water from the early season to harvest. Although the farm's seasonal average was twice the nitrite level in the incoming water, it was much below the levels found in TV3 and TV2 stations on the TSVA farm (0.23 and 0.41 mg/L, respectively; Table 14). The farm's seasonal average was almost three times lower than the level measured at MDC. This average was also about four times lower than the nitrite level reported by Hopkins and co-workers (1993) for effluent water from ponds stocked at 44 PL/m^2 (0.5 mg/L). The low nitrite levels observed throughout the growing season were much lower than the 96h LC_{50} values (8.5-15.4 mg/L) reported for shrimp (Armstrong et *al.* 1976, Wickins 1976) or the 96h LC_{50} values (532 and 756 mg/L) reported for two species of shellfish (Epifanio and Sma 1975).

Nitrate (NO_3-N)

The farm's monthly average level of nitrate in the incoming water varied between 0.26 and 0.63 mg/L, with a seasonal daily average of 0.42 mg/L (Table 18). These levels were similar to the corresponding values from the other two farms (Tables 14 and 18). As was the case for TSVA farm, the monthly average of nitrate levels in the incoming water increased from the early season to harvest. The seasonal average for the farm effluent water was 0.34 mg/L. This level was lower than the level in the incoming water. No increase in monthly averages was noticed in the SSI effluent water as was found for the other two farms. The farm's seasonal average nitrate level was much lower than the corresponding value from the MDC station (Table 14).

Total Phosphorus (TP)

The monthly average level of TP in the incoming water varied between 0.21 and 0.32 mg/L, with an increasing trend from the early growing to the harvest season (Table 18). The farm's seasonal TP average of the incoming water was 0.27 mg/L. These values were similar to levels measured in the incoming water of the TSVA farm but far below the corresponding levels from HSF. The high TP levels in the incoming water for both farms may reflect the heavy TP load into the Arroyo Colorado water from wastewater treatment plants and other sources in the area. The seasonal TP average for SSI effluent water (0.34 mg/L) was lower than the corresponding values for the MDC station and the TV2 and TV3 outlets on TSVA farm (Table 14).

pH

The farm's seasonal average of pH for the incoming water was 8.4 (Table 19); this value was similar to the level recorded for the TSVA farm. The farm's seasonal average of pH for the effluent water was 8.0 (7.4 to 8.6 range). These pH levels were within the range required by the farm's discharge permit.

Dissolved Oxygen (DO)

The farm's seasonal average of DO in the incoming water was 8.3 mg/L (2.3 to 20.1 mg/L). This average DO level was much higher than the 6.8 mg/L value of the TSVA farm (Table 13). The farm's DO monthly average in the incoming water varied between 6.7 and 9.4 mg/L (Table 19). The monthly minimum DO level varied between 2.3 and 4.1 mg/L. These low DO readings suggest that on a few occasions, the minimum DO level in the incoming water was below the standard set by the regulatory agency for the farm's effluent water (3.0 mg/L). The farm's seasonal average of DO in the effluent water was 5.7 mg/L, with a 2.7 to 8.3 mg/L range. The TNRCC permit requires the effluent water to have a 6.0 mg/L daily average of DO, with a minimum daily average of 3.0 mg/L. Although the monthly averages of DO levels in the effluent water from this farm were higher than the corresponding values from the other two farms, these levels were below the discharge permit requirements. These findings suggest that an increase in effluent DO level is needed to meet regulatory requirements.

Five-Day Carbonaceous Biochemical Oxygen Demand ($CBOD_5$)

The farm's seasonal average $CBOD_5$ in the incoming water was 4.0 mg/L. The $CBOD_5$ monthly average for October was much lower than the averages for the other three months. The CBOD, levels of the incoming water for this farm were similar to the corresponding values in the incoming water of the TSVA farm (Table 13). There was no increase in $CBOD_5$ of the incoming water from the early season to harvest. These data suggest that the river's CBOD, levels were controlled by factors other than the effluent discharge from the two farms. The seasonal daily average of CBOD, of the farm's effluent water was 1.7 mg/L, with a 1.3 to 2.7 mg/L range. This average was much lower than the corresponding value for the MDC station. The $CBOD_5$ values for SSI and the other farm were lower than the 8.5 mg/L BOD level reported by Hopkins and co-workers (1993) for shrimp pond effluent water in South Carolina. The seasonal and the monthly averages of CBOD, level for the farm's effluent water were below the 4 mg/L limit set by the permit.

CHARACTERIZATION OF INTAKE AND EFFLUENT WATERS OF HARLINGEN SHRIMP FARMS (HSF)

Water samples were collected from the farm's intake station (H1) and the discharge canal prior to the final discharge into the receiving water (H2). Table 20 summarizes the changes in TSS, total phosphorus, reactive phosphorus, and pH in these stations.

Total Suspended Solids (TSS)

The TSS monthly averages in the farm's incoming water varied between 11.6 and 24.8 mg/L, with a seasonal daily average of 18.6 mg/L (H1 station; Table 20). The seasonal average was a little higher than the corresponding value from the TSVA farm intake station. High TSS monthly averages coincided with the "brown tide" algae bloom near the farm's intake station. The July monthly TSS average in the discharge station was much higher than the other month's averages. The main reason for this high value is the salt interference in the analysis method. Since HSF's water salinity was much higher than the Arroyo Colorado, adjustment to analytical procedures was needed to ensure accurate measurements. Excluding the July average, the monthly TSS averages of the farm's effluent water varied between 73.5 and 105.2 mg/L. Although lower stocking densities were employed in this farm (12.5-19 PL/m^2), the effluent's TSS monthly average for August through October was only slightly lower than the corresponding values for TSVA farm, where stocking density of 50 PL/m^2 was employed. This finding suggests that differences in stocking densities cannot explain the relatively high TSS level in the effluent water from this farm. The farm's monthly average TSS level was over five times higher than the standard set for the SSI farm. The quantity and characteristic of the VSS in the effluent water will have to be studied further to develop an adequate TSS reduction treatment method.

Total Phosphorus (TP)

The TP seasonal average in the incoming water was 0.05 mg/L, with a 0.01 to 0.11 mg/L range (Table 20). Very little TP increase was noticed from the early season to harvest (from 0.04 to 0.08 mg/L). This level was much lower than the seasonal average for the intake of the TSVA farm (0.29 mg/L; Table 10). The seasonal average of TP of 0.15 mg/L for the farm's effluent water was over three times lower than the corresponding levels in the effluent water of the TSVA farm.

Reactive Phosphorus (RP)

The RP seasonal average in the farm's incoming water (Table 20) was very low compared with the readings from the Other two farms (<0.00 vs. 0.12 mg/L). The main reason for these differences is the fact that HSF receives its water from the Laguna Madre, while the other two farms pump water from a river that receives effluent water from municipal and industrial wastewater treatment facilities. Only a small increase in Rl? in the farm's effluent water was found. The seasonal daily average of RP in this water was only 0.05 mg/L.

pH

The pH seasonal average for the farm's incoming water was 8.4, with an 8.1 and 8.6 range (Table 20). Although the farm's water salinity was higher than for the TSVA farm, pH level was similar. The seasonal average pH level in the farm's effluent was 8.6 mg/L, with a 8.2 to 8.7 range. This pH was much higher than the corresponding values from TV2 and TV3 stations on TSVA farm (Table 13). The high "brown tide" algal concentration in the farm's effluent water was probably the main reason for these higher pH values. The pH data collected suggest that the effluent water from this farm will meet the pH limit set for the other two farms.

Five-Day Carbonaceous Biochemical Oxygen Demand ($CBOD_5$)

The CBOD, seasonal average level in the farm's incoming water was 3.7 mg/L, with a 0.4 to 10.8 mg/L range (Table 21). This level was similar to the corresponding value from the intake water of the TSVA farm. The highest monthly average value was found in September (7.3 mg/L, with a 3.1 to 10.8 mg/L range). The farm's seasonal average of CBOD, in the effluent water was 9.2 mg/L, with a 5.6 to 14.4 mg/L range. This level was over two times higher than the corresponding values in the effluent water from the TSVA farm. The farm's seasonal average value was close to the 8.5 mg/L BOD level reported by Hopkins and co-workers (1993) for shrimp pond effluent water in South Carolina. The relatively high CBOD, levels in the farm's effluent water suggest that this water was rich with dissolved organic matter and bacterial population. It is possible that the observed high level is associated with the high concentration of the "brown tide" algae in this water. Nevertheless, further studies are needed to identify the source for the relatively high CBOD, levels in the farm's effluent water. Based on the current TNRCC's water permit requirements for SSI, effective September 1, 1995, the daily average of $CBOD_5$ levels in the effluent water should not exceed 4 mg/L nor 1,513 kg (3,336 lb) a day. The farm's seasonal average of $CBOD_5$ level in the effluent water was higher than the standard set by the regulatory agency. An adequate effluent treatment facility will be needed to meet TNRCC standards.

Dissolved Oxygen (DO)

The DO levels were not recorded for the farm's incoming water during the 1994 season. For this reason, it is unclear whether the "brown tide" algal bloom affected the DO level in the incoming Water. The seasonal average of

Table 20. Changes in total suspended solids, total phosphorus, reactive phosphorus, and pH in the intake and effluent water of Harlingen Shrimp Farm (HSF) during the 1994 production season.

Period	Value	TSS[a] (mg/L)		Total P[d] (mg/L)		Reactive P[e] (mg/L)		pH	
		H1[b]	H2[c]	H1	H2	H1	H2	H1	H2
Jul.	Av.[f]	**22.0**	**207.2**	NA	NA	0.00	0.08	8.5	8.7
Aug.	Av.	11.6	105.2	0.04	0.15	NA	NA	8.3	8.5
Sep.	Av.	24.8	73.5	0.06	0.13	0.00	0.01	8.5	8.6
Oct.	Av.	15.0	93.5	0.08	0.21	0.00	0.08	8.3	8.2
Jul.-	Av.	18.6	127.7	0.05	0.15	0.00	0.05	8.4	8.5
Oct.	STD.[g]	9.9	78.6	0.03	0.05	0.00	0.10	0.1	0.2
	Max	40.0	309.0	0.11	0.25	0.01	0.30	8.6	8.7
	Min	3.0	36.0	0.01	0.04	0.00	0.00	8.1	8.2
	n[h]	16	16	11	11	8	8	17	17

[a] Total suspended solids
[b] Sampling station at the water intake station of HSF
[c] Sampling station at the water discharge outlet of HSF
[d] Total phosphorus
[e] Reactive phosphorus
[f] Average
[g] Standard deviation
[h] Number of observations

Table 21. Changes in dissolved oxygen, five-day carbonaceous oxygen demand, ammonia, and nitrate levels in the intake and effluent water of Harlingen Shrimp Farm (HSF) during the 1994 production season.

Period	Value	DO (mg/L)		$CBOD_5$[c] (mg/L)		NH_3-N (mg/L)		NO_3-N (mg/L)	
		H1[a]	H2[b]	H1	H2	H1	H2	Hl	H2
July	Av.[d]	**NA**	**4.0**	**1.2**	**11.4**	**0.02**	**0.01**	**NA**	**NA**
Aug.	Av.	NA	4.4	2.1	9.4	0.02	0.02	0.38	0.66
Sept.	Av.	NA	4.6	7.3	10.5	0.03	0.02	0.53	0.68
Oct.	Av.	NA	3.2	5.2	5.7	0.02	0.10	0.40	0.50
Jul.-	Av.	NA	4.1	3.7	9.2	0.02	0.03	0.44	0.65
Oct	STD.[e]	NA	0.9	3.2	2.9	0.02	0.06	0.15	0.07
	Max	MA	6.2	10.8	14.4	0.05	0.23	0.70	0.70
	Min	NA	0.4	0.4	5.6	0.00	0.01	0.30	0.50
	n[f]	NA	12	12	12	15	5	10	10

[a] Sampling station at the water intake station of HSF
[b] Sampling station at the water discharge outlet of HSF
[c] Five-day carbonaceous biochemical demand
[d] Average
[e] Standard deviation
[f] Number of observations

DO level for the farm discharge station (H2) was 4.1 mg/L (Table 21). This level was a little lower than the corresponding values from discharge stations TV2 and TV3 on the TSVA farm (Table 13). The DO monthly averages for the farm were lower than the limits set for the other two farms. Increased DO levels in the effluent will be needed to meet regulatory requirements.

Ammonia (NH_3-N)

The ammonia seasonal average in the farm's incoming water (HI station) was 0.02 mg/L (Table 21). There was no increase in ammonia level in the incoming water from the early season to harvest. The seasonal average for H2 was 0.03. with 0.01 to 0.23 mg/L range. The farm's ammonia levels for the intake and the effluent water were much lower than the corresponding values from the TSVA farm. The main reason for these differences was the high algal bloom ("brown tide") in the incoming and effluent water of the farm. Research conducted with this algae under a controlled environment concluded that this algal species strives on ammonia (DeYoe and Suttle 1994). The effluent ammonia levels at this farm were much lower than the 6.5 mg/L values reported by Chen and co-workers (1986, 1989) for intensive shrimp ponds in Taiwan. Ammonia levels were also much lower than the "safe level" for larvae and juvenile shrimp (1.1-1.4 mg/L) reported by several researchers (Wickins 1976, Chen and Chin 1988, Chin and Chen 1988, Wajsbrot *et al.* 1990). The farm's ammonia levels were lower than the 96h LC_{50} value range (0.4-3.1 mg/L) reported for fish (Ball 1967, Colt and Tchobanoglous 1976) and the 3.3-6.4 mg/L value range reported for marine mollusc (Epifanio and Sma 1975). Colt and Amstrong (198 1) stated that "significant growth reduction will occur in most aquatic animals at an ammonia level of 0.050-0.2 mg/L." The low ammonia level recorded for this farm is in agreement with Hopkins and co-workers (1993) which reported a daily average ammonia level of 0.08 mg/L in effluent water from ponds stocked at 44 PL/m^2 with 25% daily water exchange. It is possible that the low ammonia levels observed for both locations were a result of high algal blooms which removed any free ammonia from the effluent water. The ammonia level in the farm's effluent water was extremely low and well below the standard set by the regulatory agency for the other two farms. However, it is expected to have higher effluent ammonia levels should the farm operate under no "brown tide" algae prevalence.

Nitrate (NO_3-N)

The nitrate monthly averages in the farm's incoming water varied between 0.38 and 0.53 mg/L, with a seasonal average of 0.44 mg/L (Table 21). Although the farm's monthly averages were similar to the values found in the incoming water for the TSVA farm, the seasonal average was a little higher (0.44 vs. 0.36 mg/L). Monthly average nitrate levels in the farm's effluent varied between 0.5 and 0.68 mg/L, with a seasonal average of 0.65 mg/L. This concentration was similar to the seasonal averages of outlets TV2 and MDC on the TSVA farm's site. A relatively high level of nitrate was found in the farm's effluent although this water had a high concentration of "brown tide" algae. Possible explanation for these relatively high nitrate concentrations can be provided by recent research findings. DeYoe and Suttle (1994) found that unlike nitrite (NO_2) and ionized ammonia (NH_4^+), this algal species cannot utilize nitrate (NO_3^-). The nitrate level reported by Hopkins and co-workers (1993) for effluent water from shrimp ponds stocked at 44 PL/m^2 was about 10 times higher than the farm levels. Only high levels of nitrate (>90 mg/L) were reported to affect growth of aquatic animals (Wickins 1976). This same author reported a 48h LC_{50} value of 3,400 mg/L for juvenile shrimp. Colt and Tchobanoglous (1976) reported a 96h LC_{50} value between 1,000 and 2,000 mg/L for fish. Epifanio and Sma (1975) reported a 96h LC_{50} value between 2,600 to 3,800 mg/L for Crassostrea virginica.

SUMMARY AND RECOMMENDATIONS

The paper provides a brief review of the published information on the impact of shrimp farm effluent waters on receiving waters. Potential benefits and adverse effects on the environment and costal communities are highlighted. A large volume discharge of nutrient-rich waters from shrimp farms can result in a major negative environmental impact. Nevertheless, there is a general lack of field dam regarding the nutrient load and the quality of effluent from shrimp farms. The same is true for well-documented studies related to the ecological effects of these effluent waters. Data from literature suggest that better monitoring of selected water quality parameters in the growout ponds can reduce the farms' discharge volume. Furthermore, preliminary observations from a small-scale study conducted in South Carolina suggest that by increasing the ponds' aeration rates, water exchange can be completely eliminated. Improved aquaculture practices in terms of adequate site selection, farms' operation efficiency, feeding, feed utilization and diet formulation are only a few of the potential tools to reduce nutrient loads in shrimp farms' effluent waters, Integrated polyculture practices to reduce wasteloadings is another concept used by shrimp farmers in Southeast Asia. Under these practices, water is circulated between shrimp, fish, bivalves and macroalgae ponds to minimize effluent water discharge.

Except for a recent viral disease outbreak, effluent discharge is the major obstacle for vigorous growth of the shrimp farming industry in Texas. For the last 2 yr, the Texas Agricultural Experiment Station (TAES) has been involved in an extensive research program aimed toward

helping this industry. Intake and effluent waters of three aquaculture facilities in south Texas were monitored for about four months to cover the period between the early growout and the harvest season. Effluent characterization was provided for a high density shrimp farm (TSVA) and a low density shrimp farm (HSF), as well as an eel farm (SSI). Limited monitoring was also conducted to describe the effect of the effluent water from the high density shrimp farm and the eel farm on receiving waters. At the time that this report was prepared, only two of the farms (TSVA and SSI) were required to monitor and control six key effluent water quality parameters. These parameters were: daily discharge volume; DO: pH; TSS; ammonia (NH_3-N); and five-day carbonaceous biochemical oxygen demand ($CBOD_5$). Monitoring of other parameters (e.g., nitrite, nitrate, TP and RP) was needed for reporting only. For all three farms, the effluent pH levels were the only parameters within the limit (6-9) set by the state regulatory agency (TRNCC). The daily average effluent DO levels for all three farms were below the 6mg/L limit. Daily average effluent ammonia levels for the low density shrimp farm were much lower than the 1 mg/L limit set for the other two farms. Daily average effluent ammonia levels in the high density shrimp farm and the eel farm were generally higher than the limit set by TNRCC. The daily average effluent $CBOD_5$ levels in these two farms were lower than the 4 mg/L upper limit set by the regulatory agency. Effluent daily average $CBOD_5$ levels for the low density shrimp farm were higher than the daily average level allowed by the TNRCC. Daily average effluent TSS levels for all three farms were above the 30 mg/L limit. A very high TSS load (over 900 mg/L) can be expected in effluent waters during shrimp harvest. Extensive monitoring of the changes in TSS and VSS along a section of a drainage ditch at the high density shrimp farm suggests the following: (1) drainage ditch soil erosion is one of the reasons for the high TSS levels in the farm's effluent waters; and (2) the farm's drainage ditch served as a primary settling basin and helped to reduce effluent TSS levels.

The levels of the other nutrients in the effluent waters from the three farms were generally higher than the levels in the farms' intake waters. The limited monitoring of the receiving waters suggests that the farms' effluent water had a measurable impact only close to the farms' discharge point. No increase in nutrient and TSS levels could be detected at a distance greater than 400 m (0.25 mile) from the discharge point. Based on the data obtained from this study, a few modifications were implemented in the three farms to improve the effluent water quality. To reduce the level of TSS being released into receiving waters, the following correction steps were taken: (1) sections of the drainage ditches with high soil erosion were lined with geotextile membrane; (2) primary drainage ditches on the farm sites were deepened and widened to enhance TSS settling; (3) TSS-rich harvest waters are pumped into empty ponds where they are kept for a few days to enhance settling of particulate matter before final release into receiving waters; and (4) preliminary studies were initiated to evaluate bivalve capability to reduce TSS level in the farm effluent waters. Several studies are planned to evaluate potential methods to reduce the levels of nutrient being released into receiving waters. These studies will have three objectives: (1) determine whether increased pond aeration rates can result in lower water volume usage by the farms; (2) determine whether shrimp farms' effluent nutrient load can be reduced by altering diet formulations with no adverse effect on shrimp production; and (3) determine whether effluent ammonia and TSS levels can be reduced by adding bacterial supplement products into the growout ponds.

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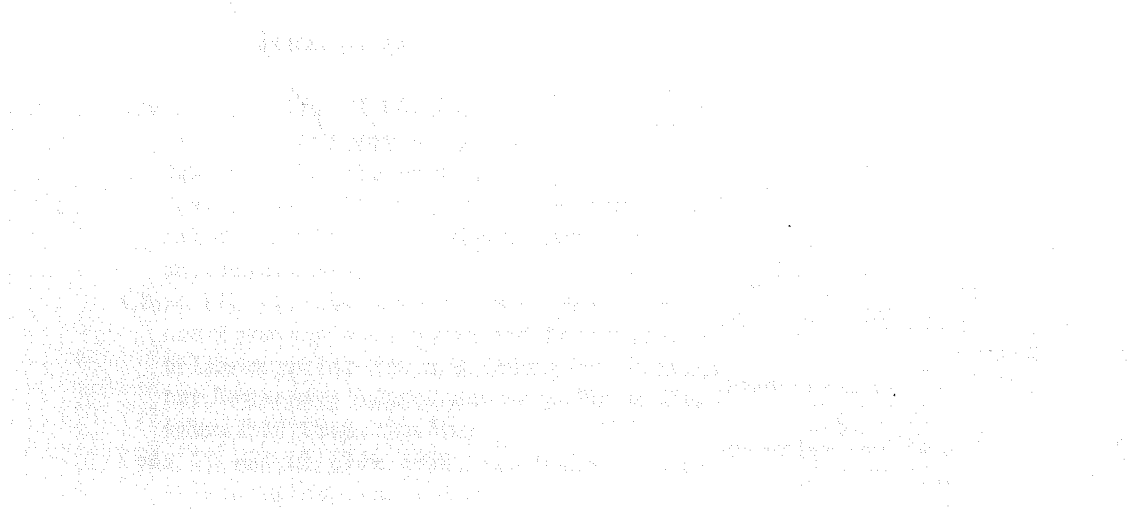

Water Quality Management by Unicellular Algae in Shrimp Larviculture Ponds

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ABSTRACT

We investigated ammonium N (NH_4-N) and phosphate P (PO_4-P) uptake by unicellular algae as a method for removing excessive nitrogen (N) and phosphorus (P) from larval shrimp rearing water and evaluated the feasibility of using algae to manage the water quality. *Tetraselmis tetrathele, Nannochloropsis oculata, Isochrysis* sp. and *Chaetoceros gracilis were* considered to be suitable algae to keep the N and P content of the rearing water low. N and P uptake of these algae from culture media and their food value to shrimp *Metapenaeus ensis* larvae were examined by

uni-algal culture or feeding experiments. Furthermore, NH_4-N and PO_4-P uptake by *N. oculata from* the larval rearing water was measured to determine the effects of algal feeding. Most of the NH_4-N and PO_4-P contained in the culture media were utilized by these algae by 16 to 27 days after inoculations. Their nutritional value, in decreasing order, was estimated to be: C. *gracilis, T. tetrathele, Isochrysis* sp.; and N. *oculata* seemed to be very low. However, a mixed feeding of N. *oculata* and an artificial diet provided better growth and higher survival rate of larvae than did each of them separately. Moreover, NH_4-N and PO_4-P content of the larval rearing water was kept lower in the mixture feeding than in the feeding of the artificial diet only. Therefore, even if the alga had low nutritional value for the larvae, adding it to the rearing water was useful in keeping the N and P content low and improving the survival rate of shrimp larvae.

INTRODUCTION

Since artificial diets for shrimp larvae have been developed and their nutritive values are estimated to be as high as live food (Kanazawa et *al.* 1982), they are used in many shrimp hatcheries in Japan. However, overfeeding of artificial diets often pollutes the larval rearing water, and nitrogen (N) and phosphorus (P) content in the water increases remarkably after a short time. Excessive N and P negatively affect larval survival and growth, so we must consider methods of coping with such pollution.

Some species of unicellular algae which are fed to shrimp larvae at protozoea (Z) and mysis (M) stages seem to be useful not only as live food, but also as water purification organisms. Unicellular algae are usually cultured in larval rearing ponds to provide good water quality for fish in many freshwater finfish hatcheries in Japan. In the current study, we investigated ammonium N (NH_4-N) and phosphate P (PO_4-P) uptake by several species of algae to evaluate the feasibility of using them to keep the water quality suitable for shrimp larvae.

MATERIALS AND METHODS

DESIGN OF EXPERIMENTS

Three experiments were carried out. The unicellular algae which were provided to the larval rearing water are expected to increase constantly in large-scale outdoor tanks and to utilize N and P from the water effectively. *Tetraselmis tetrathele, Isochrysis* sp. (Tahiti strain) and *Nannochloropsis oculafa* are *known* to exhibit constant growth in outdoor tanks (Maruyama et *al.* 1986, Boussiba *et al.* 1988, Okauchi 1988). and *Chaetoceros gracilis* is generally used as a nutritive live food (Simon 1978). Therefore, we selected these algae as appropriate species for this study.

NH_4-N, NO_3-N and PO_4-P uptake of these algae from their culture media were examined in Experiment 1. Their food value for the shrimp, *Metapenaeus ensis*, larvae at Z and M stages was estimated in Experiment 2. Then, in Experiment 3, NH_4-N and PO_4-P uptake from the larval rearing water by N. *oculata in* mixed feeding of the alga with artificial diets was measured, and the effects of the algal feeding were evaluated by shrimp growth and survival.

EXPERIMENT 1

Batch style culture was adopted for use in this experiment. The clonal uni-algal culture strains of *T. tetrathele, N. oculata, Isochrysis* sp. and C. *gracilis were grown* respectively in four 1,000 ml flat-bottom flasks containing 800 ml autoclaved medium. The medium was Guillard F (Guillard and Ryther 1962) modified to contain an adequate

Table 1. Concentrations of additives in enriched seawater media (modified Guillard F) used in experiment 1

	Trial 1	Trial 2
Seawater	100 ml	100 ml
No_3-N (as $NaNo_3$)	2.6 mg	
Nh4-N (as $(NH_4)_2SO_4$	——	2.66 mg
PO_4-P(as NaH_2PO_4)	1.6 mg	1.6 mg
$NaSiO_3 9H_2O$	3.0 mg	3.0 mg
Fe-EDTA	l.0mg	1.0 mg
$MnCL_2 4H_2O$	36.0 µg	36.0 µg
$CuSo_4 5H_2O$	1.96 µg	1.96 µg
$ZnSo_4 7H_2O$	4.4 µg	4.4 µg
$CoCl_2 6H_2O$	2.0 µg	2.0 µg
$NaMoO_4 2H_2O$	1.26 µg	1.26 µg
Vitamin B,,	0.1 µg	0.1 µg
Biotin	0.1 µg	0.1 µg
Thiamin Hcl	20.0 µg	20.0 µg

amount of NO_3-N, NH_4-N and PO_4-P (Table 1). Illumination was provided continuously by cool-white fluorescent lamps at an irradiance level of about 80 $\mu Em^{-2}S^{-1}$. The temperature was kept at about 20°C. These cultures were maintained for 20 to 27 days. Subsamplings from each flask were performed at appropriate intervals (once or twice a week) during this experiment. All samples were first filtered through glass fiber filters and the weight of cells on the filter was measured. Then, NO,-N, NH_4-N and PO_4-P content in each filtrate was determined using methods described by Strichland and Parsons (Parsons et *al.* 1984).

EXPERIMENT 2

Before Experiment 3, the food values of *T. tetrathele, N. oculata* and *Isochrysis* sp. to the shrimp larvae were estimated, comparing them with that of C. *gracilis* which is known as a nutritious algal food for shrimp larvae (Chu 1989). Vigorous nauplii which were hatched from eggs obtained from several females were randomly divided into 16 groups of 1,000 larvae each. Each group was held in a 12-L polycarbonate tank containing 10 L of filtered seawater. For each of four algal test species, four groups (T-1 to 4, N-1 to 4, I-1 to 4 and C-1 to 4) were fed *T. tetrathele, N. ocùlata, Isochrysis* sp. and C. *gracilis*, respectively.

These algae were cultured in modified Guillard F medium Cultures were harvested during the growth phase and fed to the larvae. We took into account the difference in cell size and standardized the addition of different algal species by giving nearly equal amounts by cell volume. Thus, feeding densities were set at 5-10x10^4 cells/ml for

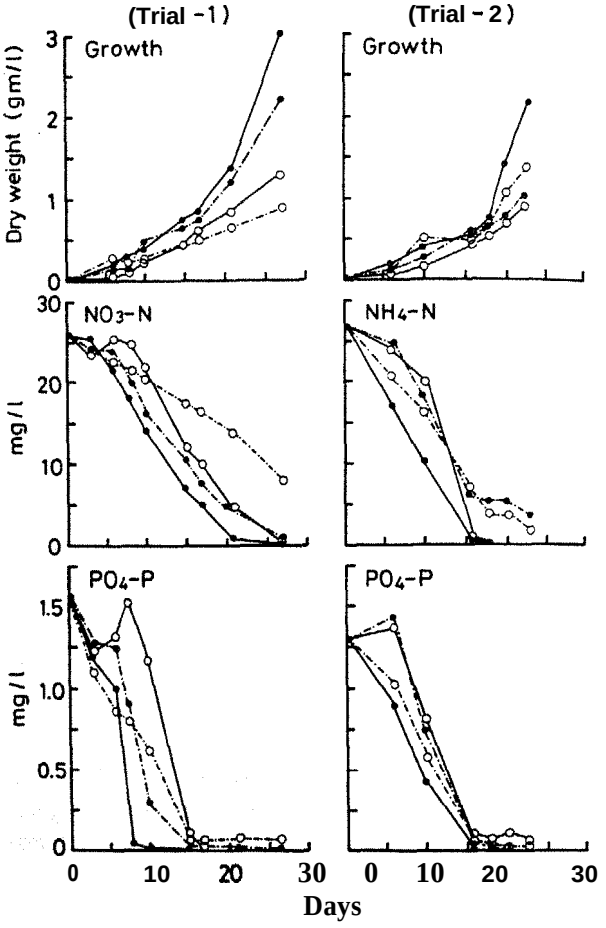

Fig. 1. Changes of NO3-N, NH4-N and PO4-P concentrations in media with the growth of Tetraselmis tetrathele (●—●) Nannochloropsis oculata (○—○), Isochrysis sp. (●---●), and *Chaetoceros gracilis* (○---○) *in experiment 1.*

T. tetrathele, 10-12x10^4 cells/ml for *Isochrysis* sp., 15-20x10^4 cells/ml for *N. oculata* and 10-14x10^4 cells/ml for *C. gracilis*. *These* densities were maintained either by lowering the water level in a tank and adding filtered seawater or by adding cultured algae. About 10 to 20% of the total volume of the tearing water was changed daily.

The experiment was continued for eight days. During the experiment, air was supplied to all culture tanks; the rearing water temperature was kept at 22 to 25°C; and illumination was provided by fluorescent lamps on 12: 12 LD cycle. At the end of the experiment, all living larvae in each tank were counted and survival rates were calculated Furthermore, 100 larvae were randomly collected from each tank and their metamorphic stages were identified by a photomicroscope, following the morphological classification of Fudinaga (1942).

EXPERIMENT 3

From the results of Experiment 2, we selected *N. oculata* as a suitable alga for this experiment. *Metapenaeus ensis* nauplii used in this experiment were hatched from eggs

Table 2. *The* survival rates and metamorphic stages of *Metapenaeus ensis* larvae fed on *Tetraselmis tetrathele*, *Isochrysis* sp., *Nannochloropsis* sp., and *Chaetoceros gracilis* at the end of experiment 2

Tank	Cell density of[a] feeding algae (x 10^4 cells/ml)	Number of[b] nauplii (N/10 L)	Number of[c] larvae (N/10 L)	Mean of survival rate (%)± s a	Metamorphic stage of larvae[d] Z3	M1	M2	M3
T-l	5 - 10	1,000	762		0	0	10	90
T-2	5 -10	1,000	701	77.5±6.7	0	0	35	65
T-3	5 - 10	1,000	883		0	0	14	86
T-4	5 - 10	1,000	752		4	0	21	79
I-l	10 - 12	1,000	691		0	0	30	70
1-2	10 - 12	1,000	712	69.6±9.0	0	0	22	78
I-3	10 - 12	1,000	818		0	0	34	66
I-4	10 - 2	1,000	564		0	0	8	92
N-l	15-20	1,000	65		60	5	0	0
N-2	15 - 20	1,000	180	11.3±4.5	70	30	0	0
N-3	15 - 20	1,000	128		52	48	0	0
N-4	15 - 20	1,000	80		80	0	0	0
C-l	10 - 14	1,000	712		0	0	6	94
C-2	IO- 14	1,000	910	81.3±8.0	0	0	12	88
C-3	10 - 14	1,000	871		0	0	0	100
C-4	10 - 4	1,000	760		0	0	23	77

a Feeding densities of *T. Tetrathele* (Ltank T-l, T-2, T-3, T-4), *Isochrysis sp.* (Tank I-1, I-2, I-3. I-4), N. oculata (tank N- 1, N-2, N-3, N-4) and C. *gracilis* (tank C-l, C-2, C-3, C-4). The densities were maintained during the experiment.

b The number of *M. ensis* nauplii acocmmodated in a tank at the beginning of the experiment.

c The number of living larvae in a tamk until the end of the experiment.

d The metamorphic states of 100 larvae collected from each tank at the end of the experiment (Z3: protoaoel stage 3, Ml: mysis 1, M2: mysis 2, M3: mysis 3).

obtained from several females. Healthy nauplii which had been reared about 6 h after hatching were collected and then randomly divided into three groups of 5,000 nauplii each. Each group was held in a 30-L polycarbonate tank (tanks AN- 1, C-l, A- 1) containing 25 L of filtered seawater provided with adequate aeration. Artificial diets and N. *oculata* were provided in tank AN-l, C. *gracilis* only was provided in tank C- 1 and artificial diet only was provided in tank A- 1. The larvae were reared from Z1 to M3 stages for seven days. The experiment was repeated using eggs obtained from other females (tanks AN-2, C-2, A-2).

Nannochloropsis oculata and C. *gracilis,* cultured in modified Guillard F medium Cultures, were harvested during their growth phases and added to each tank. During the experiment, algal cell densities in tanks AN and C were measured twice daily with a Coulter counter and adjusted to 15-20x10^4 cells/ml for N. *oculata* and 10-14x10^4 cells/ml for C. *gracilis* by lowering the water level and adding filtered seawater or by adding algae. About 10 to 20% of the rearing water in each tank was exchanged daily to remove metabolites and uneaten artificial diet. NH_4N and PO_4-P were measured every day by the same method used in Experiment 1. Larval density in each tank was estimated by counting larvae in five 500 ml samples. Survival rates of larvae were calculated at the end of the experiment. Larval growth was measured in terms of the metamorphic stage, and recorded daily by taking two samples of 10 larvae from each tank The water temperature was kept at 25°C.

RESULTS AND DISCUSSIONS

NAND P UPTAKE OF ALGAE

The results of Experiment 1 are shown in Fig. 1. All algae increased well during this experiment. On the other hand, NO_3-N, NH_4-N and PO_4-P in each medium decreased with the growth of algae in trials 1 and 2. NO_3-N was completely utilized by *T. tetrathele 21* days after the algal inoculation, and by *N. oculata* and *Isochrysis* sp. after 27 days. The NO_3-N uptake rate of *C. gracilis was* low compared with that of the other algae used, and about 8 mg/L of NO_3-N remained at the end of the culture period. NH_4-N was completely utilized by *T. tetrathele* and N. *oculata after* 16 days. In *Isochrysis* sp. and *C. gmcilis* cultures, the uptake rate of NH_4-N was high-almost equal to that of other algae-but the rate went down after the 16th to 18th day, so that 2-4 mg/L of NH_4-N remained at the end

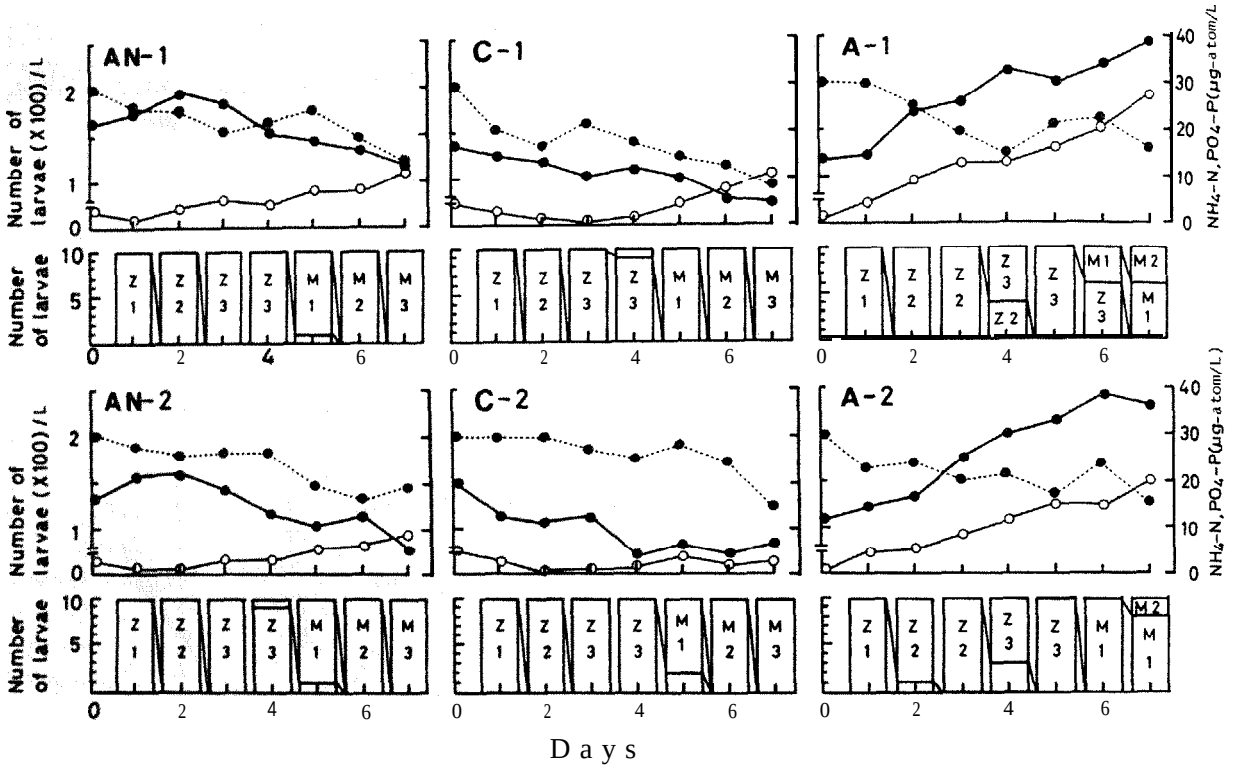

Fig. 2. Daily changes of densities (●----●) and *metamorphic stages of living* M. ensis larvae, and *daily changes of ammonium-N* (●—●) *and phosphate-P* (○—○) *concentrations in the larval rearing water in experiment* 3. *Artificial diet and N.* oculata were *provided into the rearing water in tank AN (-1 and -2). On the other hand, C.* gracilis *was provided into tank C (-1 and -2), artificial diet was provided into tank* A *(-1 and -2). Z1 shows protozoeal* stage *1, Z2: protozoeal stage 2, Z3: protozoeal state 3, M1: mysis stage 1, M2: mysis stage 2, M3: mysis stage 3.*

of the culture period. Thus, NH_4-N seemed to be more readily utilized as a nitrogen source by these algae than NO_3-N. This phenomenon was especially clear in C. *gracilis* culture. However, concentrations of NH_4-N and NO_3-N in larval rearing water under normal conditions seemed to be lawer than those of the medium used in this experiment. Therefore, even if C. *gracilis were* added to the larval rearing water, NH_4-N and NO_3-N were utilized effectively and their concentrations were kept at low levels. Furthermore, PO_4-P was almost completely utilized 16 days after beginning the culture regardless of algal species. Therefore, the algae can be effective in removing excessive N and P from the shrimp rearing water.

FEED VALUES OF ALGAE

The survival rates and metamorphic stages of *M. ensis* larvae at the end of Experiment 2 are shown in Table 2. The survival and development rates of larvae fed on N. *oculata* were obviously inferior to those of larvae fed on other algal species. Most larvae in tank N (-1 to -4) died at Z1 and Z2 stages, and the few surviving larvae were Z3 and M1 stages at the end of the experiment. Therefore, N. *oculata* seemed to be inadequate as a food organism for the shrimp larvae. The principal reason for this result seems to be that *N. oculata* is too small to chew and has a hard cell wall, so that larvae are unable to digest it.

On the other hand, the survival and development rates of larvae fed on *T. tetrathele* or *Isochrysis* sp. were slightly lower and slower than those of larvae fed on C. *grucilis. T. tetrathele* is bigger than N. *oculata* and has a relatively thin cell wall, while *Isochrysis* sp. does not have a cell wall, so that larvae seem to digest them easily. However, these algae have been found to contain little eicosapentaenoic and docosahexaenoic acid in comparison with *C. gracilis (Helm* and Laing 1987, Okauchi 1988, Su *et al.* 1988). These fatty acids were shown to be essential for *Penaeus japonicus* larvae (Kanazawa *et al.* 1978). Therefore, the nutritive values of *T. tetrathele* and *Isochrysis* sp. seem to be inferior to that of C. *gracilis.*

EFFECTS OF MIXED FEEDING OF ALGAE WITH ARTIFICIAL FEED

We chose N. *oculata as the* alga to be added to the larval rearing water in Experiment 3 so as to minimize the food effect and make the effect of N and P reduction clear. Daily changes in densities and metamorphic stages of larvae in each tank are shown in Fig. 2. Other results of Experiment 3 are presented in Table 3. Larval densities gradually decreased during this experiment, and there was no significant difference in terms of changes in larval den-

sities between each tank. Mean survival rates of tanks AN, C and A in two trials were 71.3%, 57.5%, and 63.8%, respectively. On the other hand, the development of larvae in tank A was obviously slower than that of the other tanks. Although all larvae in tanks AN and C had already metamorphosed into mysis stage 3 at the end of the experiment, more than 50% of the larvae in tank A were still in mysis stage 1. NH_4-N and PO_4-P concentrations in tanks AN and C decreased from the beginning of the experiment, and they remained at low levels. Conversely, these concentrations in tank A gradually increased, and were about three to eight times for NH_4-N and about two to six times for PO_4-P in comparison with concentrations in other tanks at the end of this experiment.

As confirmed in Experiment 2, the nutritional value of N. *oculata* was low and that of C. *gracilis was* high. However, the survival rate of larvae in tank AN was highest of all, and the development rate was almost equal to that of larvae fed on C. *gracilis.* One reason for such results could be that the artificial diet used in this experiment seemed to be nutritious enough for larvae, but it polluted the water and seemed to create an unsuitable environment for the larvae. On the other hand, N. *oculata* effectively utilized NH_4-N and PO_4-P from the water, so that the water quality remained appropriate for larvae in spite of the addition of artificial diet. Therefore, suitable conditions in regard to both nutrition and water quality were maintained by mixed feeding *of N. oculata* and artificial diet.

Table 3. Feeding concentrations of algae (*N. oculata* or *C. gracilis*) and artificial diet, survival rates and developments of larvae, change of NH_4-N and PO_4-P concentrations in experiment 3

Tank	Feeding concentrations of alga and/or artificial diet (gm/day/tank)	a Number of nauplii (N/25L)	b Number of larvae (N/25L)	Survival rate (%)	c Metamorphic stages of larvae			d NH_4-N concentration (μg - atom/L)		e PO_4-P concentration (μg - atom/L)	
					M1	M2	M3	Beginning	End	Beginning	End
AN-1	15-20x104(N. oculata) : 1	5,000	3.375	67.5	0	0	10	22.5	13.4	4.0	12.2
AN-1	15-20x104(N. oculata) : 1	5,000	3,750	75.0	0	0	10	17.8	5.2	3.2	9.5
C-1	10-14x10⁴(*C. gracilis*) : 0	5,000	2,550	51.0	0	0	10	18.1	4.8	5.2	10.8
C-2	10-14x10⁴(*C. gracilis*) : 0	5,000	3,200	64.0	0	0	10	20.0	7.5	5.4	3.5
A-1	0 : 1	5,000	3,125	62.5	6	4	0	13.6	39.2	1.5	28.3
A-2	0 : 1	5,000	3,250	65.0	8	2	0	11.8	36.8	1.2	20.0

a The number of *M. ensis* nauplii accommodated in a tank at the beginning of the experiment.
b The number of living larvae in a tank until the end of the experiment.
c The metamorphic stages of 10 larvae collected fromeach tank at the end of the experiment (M1: mysis 1, M2: mysis 2, M3: mysis 3).
d NH_4-N concentration of the larval rearing water in each tank at the beginning and end of the experiment.
e PO_4-P concentration of the larval rearing water in each tank at the bginning and end of the experiment.

EFFICIENT LARVAL REARING TECHNIQUES USING ARTIFICIAL DIETS AND UNICELLULAR ALGAE

The use of artificial diets should increase in popularity and those of high quality which are nutritious and almost insoluble in the rearing water will undoubtedly be developed in the near future. However, shrimp larvae, especially from protozoeal to mysis stages, are very sensitive to water pollution and nutrient deficiency. Furthermore, adequate change of the rearing water is difficult without damage to larvae in large outdoor ponds. Therefore, water pollution by shrimp metabolites and uneaten artificial food will remain a serious problem.

We found that N. *oculata was* useful in removing excessive NH_4-N and PO_4-P from the larval rearing water. Other algae which were used in this study utilized NH_4-N and PO_4-P as effectively as *N. oculata.* Nutritional values of the other species,in descending order, were: *C. gracilis, T. tetrathele* and *Isochrysis* sp.

Judging from these results, if we added these algae instead of N. *oculata* to artificial diet, the larvae

could eat and digest both algae and artificial diet in a suitable environment. Therefore, maintaining unicellular algae at a suitable density in larval rearing ponds is a useful culture technique. Unicellular algae have been studied mainly as food organisms since the development of large-scale production of *P. japonicus* by Fudinaga and Kittaka (1966, 1967).

Further studies are needed on the role of unicellular algae in water purification and a suitable system of water quality management using algae should be developed.

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Environmental Factors Influencing Clam Culture on Sandy Shores

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ABSTRACT

Artificial seed productions of the Japanese surf clam *Pseudocardium sachalinensis* and the poker-chip venus *Meretrix lamarckii are* carried out at several prefectural hatcheries, where a couple of million juveniles 3 mm in shell length are produced at each hatchery. Usually, the bulk of the juveniles are released directly at sandy shores, but this has not been successful. Two approaches are being used for future success in clam mariculture on sandy shores. One is nursery culture in natural conditions. It is necessary to grow clams to a larger size because 3 mm juveniles are moved by wave action. The experimental field nursery culture of the Japanese surf clam *P. sachalinensis* is performed in an artificial pond fenced in by iron plates. The pond will protect the juveniles from waves and help them grow larger. Water exchange and food supply in the pond will be sufficient for the growth of clams. Another is the prediction of movement of the clam seed. Dispersion and migration are very significant factors in the release of clam juveniles. On sandy shores, movement by waves is most important for clams. Numerical models for the on-offshore movement in a transection of beach are developed on the basis of hydrodynamics. The availability of the models is recognized in comparison with the field survey and the flume experiment.

INTRODUCTION

In Japan, the technology of clam mariculture, especially in growout and nursery culture, on sandy shores is not advanced in comparison with seed production. On sandy shores, it is very difficult to carry on intensive culture under completely artificial control because the wave action may wash and disperse the clam seeds. For exposed, high energy sandy beaches which have abundant primary productivity (Brown and McLachlan 1990, Adachi et *al.* 1994), extensive culture to utilize the shallow nutritious water is suitable. In this regard the supplement of artificial seed for natural resources seems to be effective for stabilizing the harvest. Actually, the artificial seed productions of the Japanese surf clam ***Pseudocardium sachulinensis*** are ***carried*** out at several prefectural hatcheries on the northern Pacific coast of Japan. Most of the hatcheries can produce a couple of million juvenile clams 3mm in shell length. Considering the cost and the time, this size is maximum as far as feeding live algae in land-based tanks. Usually, the bulk of the hatchery-reared juveniles is released in the natural environment directly, but that has not been successful. The. size of the seed is too small to stay at the released point. Thus, it is necessary to grow them to a larger size in the nursery system. In this article, we introduce studies for the future success in clam mariculture on sandy shores. One is nursery culture in natural conditions. Another is the prediction of movement of the clam seed.

PRODUCTION OF CLAMS

The abundant clam resource is the result of a dominant year-class in the variance of the natural population. Since 1987, landing of the poker-chip venus *Meretrix lamarckii* at Kashima-nada in Ibaraki Prefecture, Japan, is ea.300 kg/boat/day. It is worth approximately 200 to 300 thousand yen (equivalent to US$2000-3000).

The location of Kashima-nada in Ibaraki Prefecture and the study sites of both the experimental nursery culture and the field survey of the distribution of clams are shown in Fig. 1. Fig. 2 shows the annual landings of both the Japanese surf clam *P. sachulinensis* and the poker-chip venus *M. lamarckii* in Ibaraki Prefecture on the Pacific Ocean (Maoka 1993). The landings apparently fluctuated from year to year, and the standing stocks of these clams also similarly change. At the lower level of the smaller year-class population, the landing was less than 1% of the highest level.

The environmental factors affecting the survival, movement and dispersion of the early stage of clams in their natural condition are amount of food, water quality and clam movement by water current and wave action. The movement by waves and currents is the most severe problem for the early stage of clams on sandy shores.

POND NURSERY CULTURE

Whenever clam seeds are planted in the natural envi-

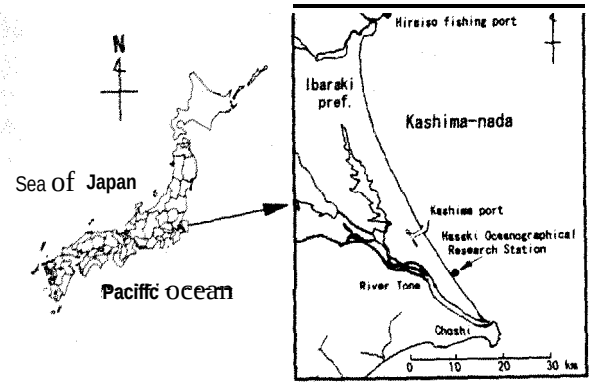

Fig. I. Location of the Hiraiso fishing port and the Hasaki Oceanographical Research Station along the coast of Kashima-nada in Ibaraki Prefecture.

ronment, some protective device from waves, predators and other factors is needed. In commercial clam species of the world, various manners of nursery systems have been adopted, such as net pen, suspended bucket as in sea-based systems and raceway tanks, and upflow and downflow systems as in laud-based systems (Manzi and Castagna 1989).

In our study, experimental field nursery culture of the Japanese surf clam *P. sachalinensis* was performed in an artificial pond fenced in by steel plates. Fig. 3 shows the pond constructed inside of the Hiraiso fishing port in Ibaraki Prefecture. Each part of the plate was connected by bolts and nuts, and strengthened by angle steel, The length of each side was 2.8 m, and the height of the wall was about 1.2 m from the seabed. Water depth around the pond changed from 0.1 m to 1.5 m due to the tide level.

Figure 4 shows a rough sketch of the pond and the disposition of wave gauges, thermometers inside and outside of the pond, and dam recorder. The wave gauge utilized was a diaphragm-type pressure gauge connected airtightly to the PVC pipe. Seawater was exchanged through the openings of the walls. Nylon screens of 3.6mm mesh were attached to the openings in order to prevent the juvenile clams from passing through. Plastic filtration materials like sponge gourds were installed on the top of the pond to protect clam seeds from the turbulence of waves. Initially, 478 thousand juveniles which had been produced at the Ibaraki Seafarming Association were planted in the pond on 4 July 1995. Average shell length was 2.8 mm at the start. At the same time, juveniles were also cultured in buckets with sand, suspended at the center of the port. This work was carried out cooperatively with the Ibaraki Prefectural Fisheries Experimental Station.

The tide levels and water temperature on the inside and outside of the pond from July 25 to August 30, 1995, are demonstrated in Figure 5. Higher temperatures more than 28°C were observed at ebbs in the spring tide. Duration of high temperature, over 26°C, continued no more than 12 h even on hot sunny days, because the wave absorber also played the role of a sunshade. As the Japanese surf clam survived and grew at 28°C, this temperature was not fatal to the clam throughout the experiment.

The change of water level measured by the wave gauges indicated that the exchange rate of seawater by waves was much greater than that by tides. Furthermore, a cumulative exchange of seawater in a day corresponded to about 50 m in height of the water column in the pond. In other words, daily exchange of water reached 30-40 times the volume of the pond. The water flow of the pond was analyzed from the measurement of the currents. Inward currents of the upper openings were about 50 cm/sec, and, even on the bottom, current velocity of 1 or 2 cm/sec was observed in calm conditions. These facts show that the structure of the pond was enough to transport phytoplankton and provide food to the clam seed.

Figure 6 shows the survival and the growth of the juve-

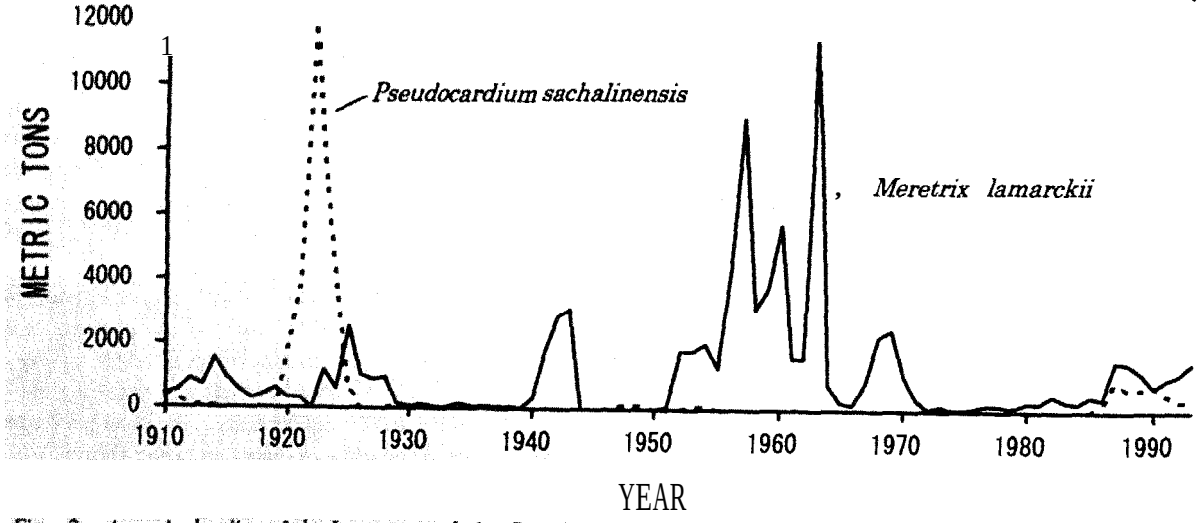

Fig. 2. Annual landing of the Japanese surf clam Pseudocardium sachalinensis *and the poker-chip venus Meretrix* lamarckii *in Ibaraki Prefecture, Japan.*

nile Japanese surf clam. Approximately 107 thousand clams were yielded 77 days later, and the average shell length reached 10.4 mm. This showed rapid growth comparable to that of the natural population and the suspended culture. On the other hand, apparently low survival of the clams was attributed to the extremely high density of the planted clams and predation by paperbubbles Philine argentata. In the former, initial density of the seed clam was about 100,000 individuals/m^2. In an extremely dense state, clams are not able to feed and keep their niche. The survival rate will increase in appropriate density. On the other hand, the invasion of predators, especially carnivorous mollusks such as the paperbubble (Philinidae), the moonsnail (Naticidae), and the starfish, is the most severe problem in field nursery systems.

Fig. 3. Photograph of the nursery pond at the Hiraiso fishing port.

PREDICTION OF CLAM MOVEMENT

On sandy shores, the passive movement by waves is most significant for clams. In fact, onshore-offshore movement of clams with rapid change of profile (Higano et *al.* 1993) and long distance transportation of released clams were reported by Shimura and Honma (1971). The purpose of this study is the development of a numerical model by computer simulation for the prediction of clam movement in the wave field. The numerical models (Kuwahara and Higano 1994a, b) for on-offshore movement in a transection of beach were constructed as a result of the hydrodynamics and the mechanics of the clam. The model consisted of three main calculation steps based on physical processes. The validity of the model was examined in comparison with the real distribution of the field survey and the flume experiment.

The first calculation step was the wave field in the on-offshore direction including the surf zone at an optional beach profile, with the time-dependent mild slope equations (Watanabe and Maruyama 1984). The second was the clam movement by one wave. The moving distance during a period of the wave is calculated using the equation of motion. And then, the moving distance by a wave train was calculated by superposing of the distance by one wave.

Figure 7 shows the components of forces acting upon a clam, such as gravity, frictional resistance, mass force and other factors. In the model, the shape of the clam was assumed to be a sphere. The equation of motion can be expressed as follows:

$$M du_s/dt = du_b/dt + C_M m d(u_b-u_s)/dt + 1/2 C_D A \rho_w |u_b-u_s| (u_b-u_s) - (M-m) g \sin\beta - \mu_r g (M-m) \cos\beta u_s/|u_s|$$

where M denotes mass of clam ($1/6\pi\rho_s D^3$, ρ_s: specific gravity of clam, D: shell length of clam); m: mass of water corresponds to the volume of the clam ($1/6\pi\rho_w D^3$, ρ_w: specific gravity of water); u_b: water velocity at the bottom by waves; u,: velocity of clam movement due to water veloc-

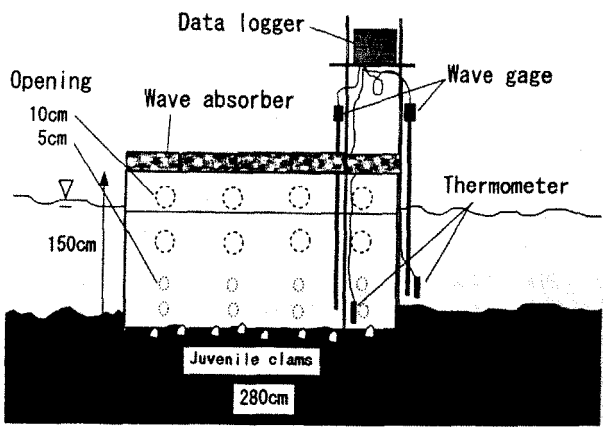

Fig. 4. Sk-etch of the nursery pond and the disposition of wave gauges, termometers, data recorder and wave absorbers.

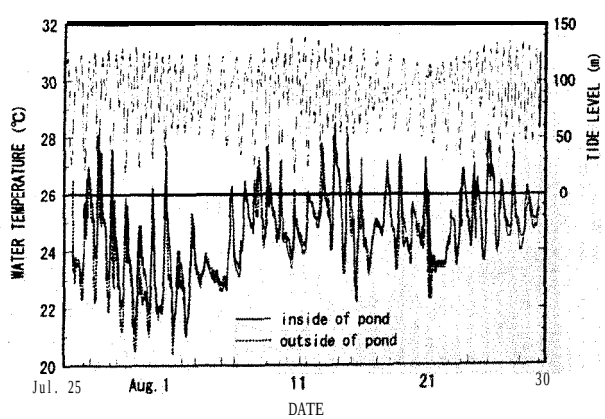

Fig. 5. Change of water temperature and tide level inside and outside of the pond.

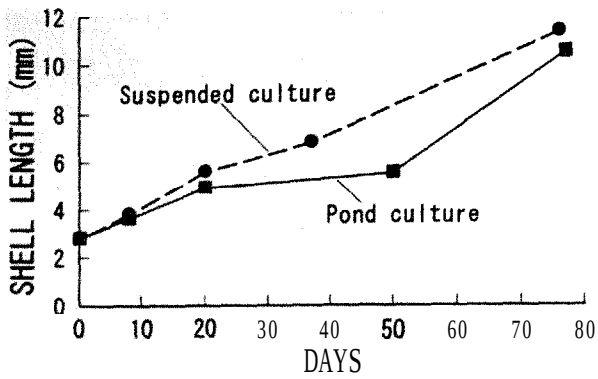

Fig. 6. Growth of the Japanese surf clam Pseudocardium sachalinensis *planted in the pond and the suspended buckets at the Hiraiso fishing port.*

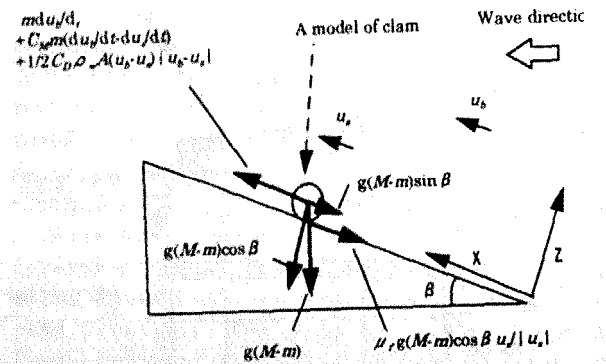

Fig. 7. Clam movement model by wave. The explanation of forces connected with the clam movement are shown as follows:

mdu_b/dt: the force caused by the pressure gradient of waves

$C_m m(du_b/dt-du_s/dt)$: the mass force caused by the relative movement between bivalve and fluid

$1/2C_D\rho_W A(u_b-u_s)|u_b-u_s|$: the drug force caused by the relative velocity

$g(M-m)\sin\beta$: the component of gravity parallel to the *seabed surface*

$-\mu_s g(M-m)\cos\beta u_s/|u_s|$: the frictional resistance force caused by sliding of the bivalve on the seabed

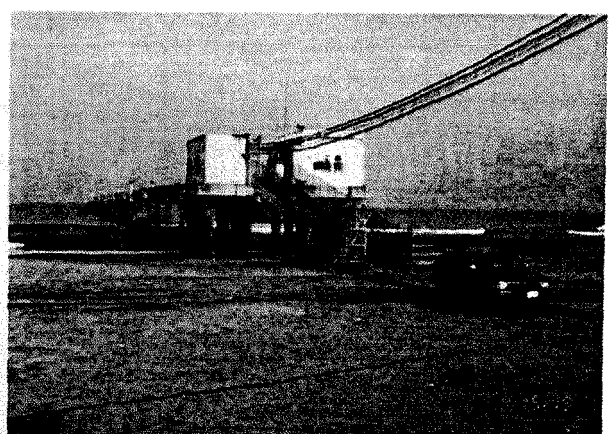

Fig. 8. Photograph of the Hasaki Oceanographical Research Station of the Port and Harbor Research Institute.

ity; u_b, C_M, C_D: coefficient of apparent mass force and drag force, respectively; A: area of the clam that projects; and μ_f: the frictional resistance coefficient.

In the field survey, sampling was carried out at intervals of 10 m along the research pier of the Hasaki Oceanographical Research Station (Fig. 8), Port and Harbor Research Institute, Ministry of Transport, on June 23, 1987, just after a storm. Figs. 9(a) and 9(b) show the distribution of bivalves and the beach profile, respectively. It clearly shows that equilateral venus *Gomphina melanaegis* was accumulated at the bottom of the trough, 200 m offshore from the shoreline.

In the calculation of the model, the values of variables are given in Table 1. It is assumed that the wave condition was moderate and the physical characteristics of the clam corresponded to young *G. melanaegis*. Figure 9(c) shows the result of the numerical simulation. The vertical lines indicate the periodic movement of clams in the calculation. It was assumed that the clams were placed on the seabed at intervals of 10 m, and the beach profile was the same as Figure 9(b). The clams gradually accumulated at the bottom of the trough. Figures 9(d) and 9(e) show the calculated distributions of the model clams 10,000 sec after the start of the calculation, for different shell length and specific gravity.

Another means to examine the validity of the model is the comparison between the laboratory experiment and the calculation. The experiment was carried out in the flume tank with a plunger-type wave generator at the National Research Institute of Fisheries Engineering. The tank and set of the experiment are shown in Figure 10. Initially, juveniles of the Japanese surf clam *P. sachalinensis* were placed on the sand bed at intervals of 40 cm from the shoreline to the offshore end of the bed along the beach profile which already had wave action for 2 h. After generating waves for 15 min, the juveniles were collected from the sand bed with a siphon at intervals of 10 cm.

Figure 11 shows the distances of clam movement in the flume experiment and the calculation, respectively. The juveniles were accumulated approximately 1 m and 5.5 m from the shoreline. The values of variables in the calculation are given in Table 2. In the calculation, the clams were accumulated 1.1 m and 6.5 m from the shoreline. Both the experiment and the model showed the divided areas in which the clams moved onshore and offshore. The calculated results also coincide with the flume experiment for the Japanese surf clam *P. sachalinensis*.

In the numerical model, it is difficult to consider the biological aspects such as burrowing behavior, shell shape and other factors. In our model, the coefficients C_D, C_M, μ_{fs} and μ_{fd} express their characteristics inclusively, and we adopted C_D and C_M as 0.5 for both G. *melanaegis* and *P. sachalinensis*. From the oscillational flow tank experiment, Yamashita et *al.* (1995) showed that the C_D and C_M

Table 1. Values of the physical characteristics of waves and clams adopted in the simulation for the field survey

Wave height	H_0 = 1.5m
Period	T = 7.0sec
Shell length	D = 20mm
Specific gravity of shell	ρS = 1.8
Coefficient	
of mass force	C_M = 0.5
of drag force	C_D = 0.5
Frictional resistance coefficient	
static	μ_{fs} = 1.0
dynamic	μ_{fd} = 0.5

of *P.sachalinensis* were 0.5 and 0.1, respectively. Each clam species has different characteristics, thus corresponding to proper values of the variables. It is necessary to determine appropriate values for target species.

CONCLUSIONS

From the results of the field experiments of the nursery pond, it is evident that the pond has a possibility to work well as an intermediate growth area. Therefore, we will propose the larger scale pond near the shoreline as one of the methods of clam culture on exposed sandy beaches. It has the advantage of the utilization of abundant phytoplankton in the surf zone and water intake utilized by wave energy. The problems pointed out are seeping fresh land water, storm damage, accumulation of sand and invasion of predators. The numerical model can predict the movement of released clams and also natural populations. As a further step, however, the model must be applied to a superficial field. Further studies are necessary for commercial culture on sandy shores.

ACKNOWLEDGMENT

We greatly appreciated the assistance of the staffs of both the Ibaraki Prefectural Fisheries Experimental Station and the Port and Harbor Research Institute in performing the cooperative study on the development of a new nursery culture system and the distribution of clams in the surf zone.

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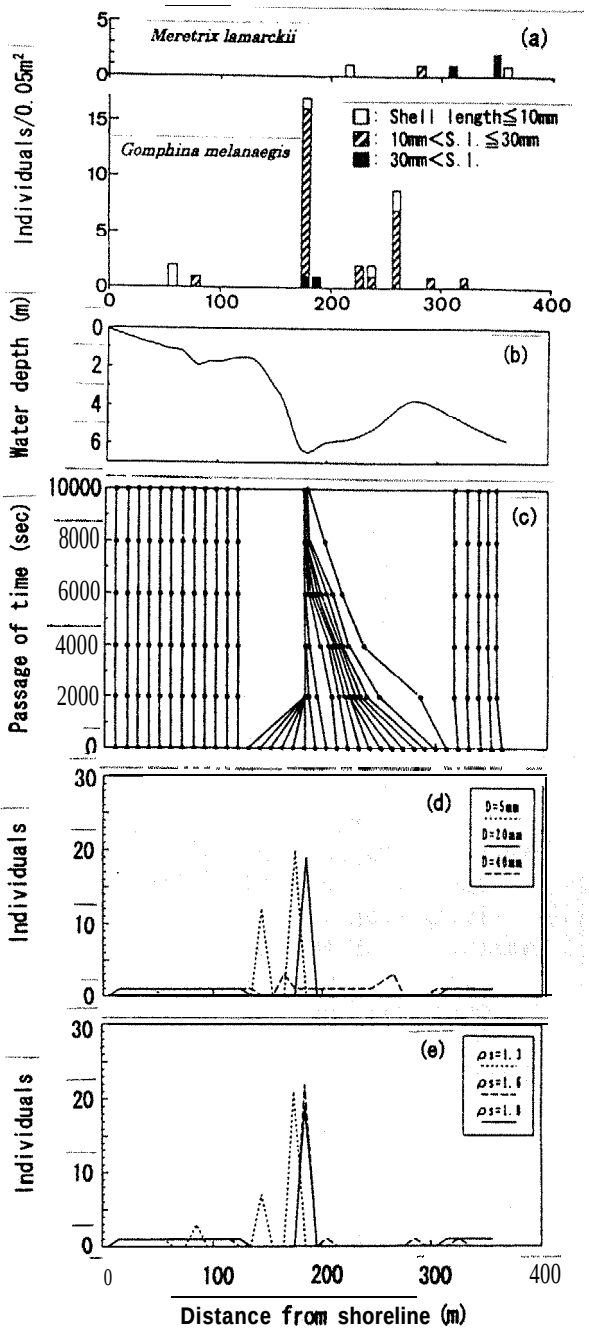
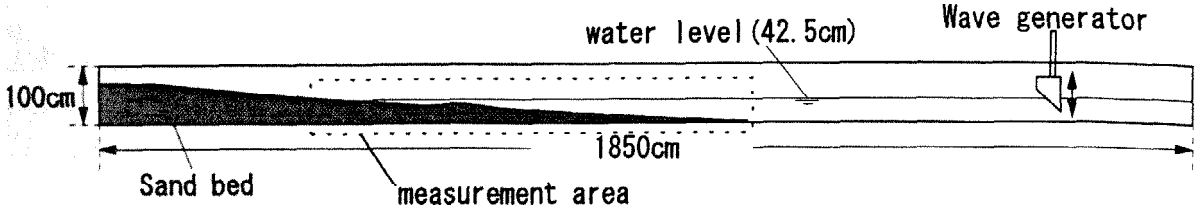


Fig. 9. Beach profile (b) and distribution of clams (a) at Hasaki Oceanographical Research Station. The numerical model was adapted to the distributions above. The change of position of clams (c) according to the passage of time. The calculations of different shell length (d) and specific gravity (e) of clams after **10,000 sec.**



Fig, 10. Schematic illustration of the wave generation tank setting the experiment of the movement of the Japanese surf clam.

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Table 2. Values of the physical characteristics of waves and clams adopted in the simulation for the flume experiment

Wave height	H_0 = 7.0cm
Period	T = 1.0sec
Shell length	D = 8mm
Specific gravity of shell	ρS = 1.21
Coefficient	
of mass force	C_M = 0.5
of drag force	C_D = 0.5
Frictional resistance coefficient	
static	μ_{fs} = 0.55
dynamic	μ_{fd} = 0.3

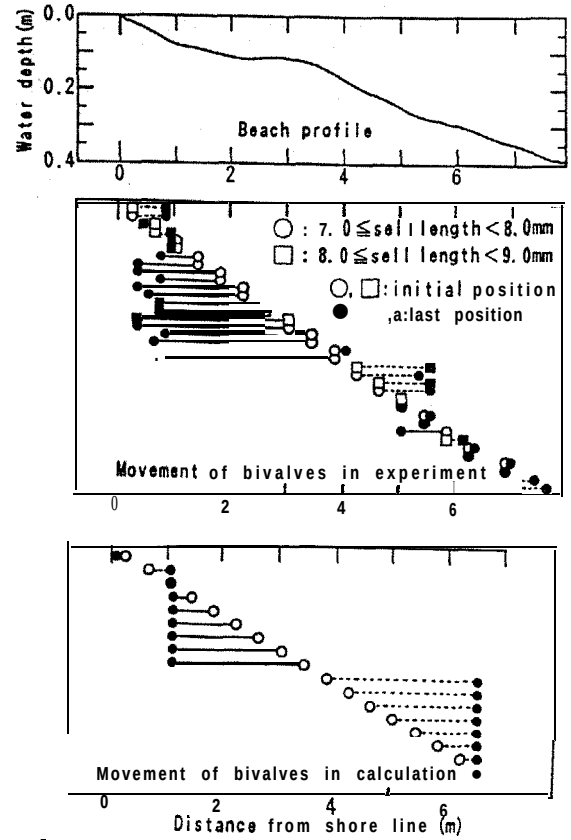

Fig. 1 I. Comparison between results of the experiment and the calculation in wave of erosion type.

A Strategic Approach to Carrying-Capacity Analysis for Aquaculture in Estuaries

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ABSTRACT

Estuaries are coastal watercourses that are subject to both marine and riverine influences. Their principal hydrographic controls are morphology, tides, freshwater inflows, meteorology, and density currents. The propagation of tides and the distribution Of salinity are important indicators of circulation in an estuary. Circulation in particular imposes a limit on the ability of an estuary to assimilate wastes without degrading its water quality. This is an important constraint on concentrated aquaculture operations that circulate water, since these produce a large volume of wastewater and also require a supply of uncontaminated water. A general procedure is outlined for determining the "carrying capacity" of the estuary. This requires (1) specification of the water quality parameter(s) that form the basis of water quality evaluation, (2) determining the parameter value(s) of acceptable water quality, (3) development of a water quality model appropriate for the estuary, and (4) establishing the conditions that are critical for water quality.

The water quality model is central to the procedure: it is a combined hydrodynamic and mass balance calculation, designed to reflect the space-time scales controlling the water management problem. Its development requires an extensive base of field data. The model is applied to predicting the water quality regime that would result under a hypothetical distribution and volume of wasteloads. The largest volume of wasteloads that results in water quality equal to the level judged acceptable under critical conditions is the assimilative capacity. It is important to note that assimilative capacity is a function of position in the estuary, and depends upon both local and larger scale hydrography. Single values of "carrying capacity" or "flushing time" applied to an entire estuary are of little use. A case study is presented of shrimp aquaculture in Golfo de Fonseca, Central America. A preliminary analysis of the Operations around Estero Pedregal is performed using a one-dimensional model, to illustrate the kinds Of analyses that can be carried out and the types Of results that can be obtained. These results indicate that shrimp aquaculture in this area is already approaching a level of being self-limited.

INTRODUCTION

THE ESTUARY SETTING

Estuaries are watercourses that occur on the fringe of the sea. An estuary is therefore influenced by both terrestrial and oceanic processes, and is transitional between a purely riverine system and a purely marine system. An estuary is generally considered to have the following properties:

- coastal waterbody
- semi-enclosed
- free connection to open sea
- influx of seawater
- influx of freshwater

The biochemical functioning of an estuary, including its ability to assimilate wasteloads, is governed largely by circulation processes which determine the capacity for dilution and the intensity of mixing. Circulation in estuaries is generally determined by the following hydrographic features:

Morphology-the physical dimensions and shape of the system. The trajectories of flow are strongly controlled by the distribution of deeps, barriers and shoals, by the configuration of the shoreline, and by bathymetry.

Tides-the movement of water in the oceans in response to differential gravitational accelerations by celestial objects, viz. the moon and sun. Tide propagates into an estuary through the mouth or main inlets, being attenuated and lagged by friction, but amplified by reflection from the convergence of the shoreline,

Freshwater inflow-the supply of freshwater into the bay. Dilution of seawater by inflow is responsible for es-

tablishing a salinity gradient across the estuary.

Meteorological forcing-the effects of winds and pressure systems on the estuary, These include generation of short-crested windwaves, development of large-scale internal circulations within the bay and wind tides, including storm surges.

Density currents-the net movement of water forced by the horizontal gradient in water density (itseif a consequence of the salinity gradient). These currents are largely responsible for the high dispersion in an estuary, and are particularly sensitive to water depth.

Turbulent diffusion-the combined effect of small- and large-scale water movement that results in mixing out gradients of concentration. Turbulence is especially important in determining the rate of dilution of pollutants, and dispersion of drainage plumes from aquaculture operations.

The hydrographic characteristics of an estuary, or a segment of an estuarine system, can be judged by determining the relative importance of these factors, which will vary with external conditions, with season and with position in the estuary. There is usually a clear zonation in morphology and water quality with distance from the sea, from deep, saline, well-aerated watercourses near the main inlet to the sea, to shallow, brackish, poorly flushed systems in the upper reaches. Indeed, one of the important ecological features of estuaries is the range of habitats created by this zonation in morphology and water quality.

The management of estuaries involves being able to determine the effect on estuary circulation, on concentrations of constituents in solution or suspension, or on elements of the estuary dependent upon these (such as biologicd communities), that results from a specific event or external control. This general statement includes a wide range of causes and effects, both natural and manmade: discharge of wasteloads, spills of hazardous or toxic substances, floods, reductions of freshwater inflow, construction of reservoirs, shoreline development, channelization, installation of hurricane barriers, alterations in land use in the watershed, and so on. Aquaculture operations that employ estuarine water are dependent upon the quality of that water. Moreover, these same operations are capable of impacting the quality of the estuary, directly by the discharge of effluent and indirectly by modifying circulation processes. While these aspects of an aquaculture operation are individually similar to other human activities in estuaries, their combination creates a novel management problem. The large areal scale of aquaculture operations, the great volumes of flow involved, and the variety of biochemical constituents of importance mean that aqua&tune has the potential for widespread, deleterious impacts on its estuarine environment. A central question in aquaculture development is bow extensive an operation can be installed in an estuary without driving the estuary quality below some minimum level. This is referred to as assimilative capacity, or carrying capacity, Of the estuary.

EVALUATION OF ESTUARY ASSIMILATIVE CAPACITY

The problem of aquaculture development requires a quantitative evaluation of water quality in the area of the proposed aquaculture operation, as measured by the concentrations of waterborne constituents. This requires quantitative cause-and-effect relationships between the alterations to the environment associated with aquaculture, and the resulting constituent concentrations. The general causal controls on estuary water quality are shown in Fig. 1. The fundamental feature of the estuarine environment, in contrast to lakes or rivers, is the central role of hydrodynamic processes in determining constituent concentrations.

While the determination of cause-and-effect can be based entirely upon data collection and analysis, this requires an extensive and costly data collection program. Moreover, many management situations necessitate that a human activity be evaluated before it is implemented. In the present context, the potential impact of aquaculture must be evaluated in advance, to support planning and management. The standard methodology is to apply a predictive model. Today, these models are numerical solutions to the equations of momentum and mass conservation, performed on a digital computer.

There are two aspects to the problem: (1) the effect of aquaculture on water quality in the area, especially resulting from waste discharges from the operation, and (2) if the estuary is to be used as a water supply, the suitability of the quality of that water, especially how that water quality is influenced by the anticipated wasteloads from the aquaculture operation itself and from other wastewater discharges in the region. Therefore, a model is needed of space-time distribution of concentration of controlling parameters in the estuary (Fig. 1). The concentration of a constituent is governed by transport processes (including mixing) and kinetic processes, so the model must include a determination of hydrodynamic transports as well as a mass balance of the water quality constituents. This is true whether the watercourse is a river. lake, aquifer, oiestuary. For an estuary, however, the complex geometry and complicated hydrodynamics make model formulation especially difficult. For this reason, the special topic of estuary modeling has long received concentrated attention, and there is extensive literature on the subject (Ward and Montague 1996). Also, this is why the hydrography of an estuary must be understood in order to evaluate its water quality.

Fig- l represents reality, which the model seeks to simulate. Detailed discussion of modeling strategy lies beyond the scope of this brief paper, though several observations are in Order. The question in model selection and develop-

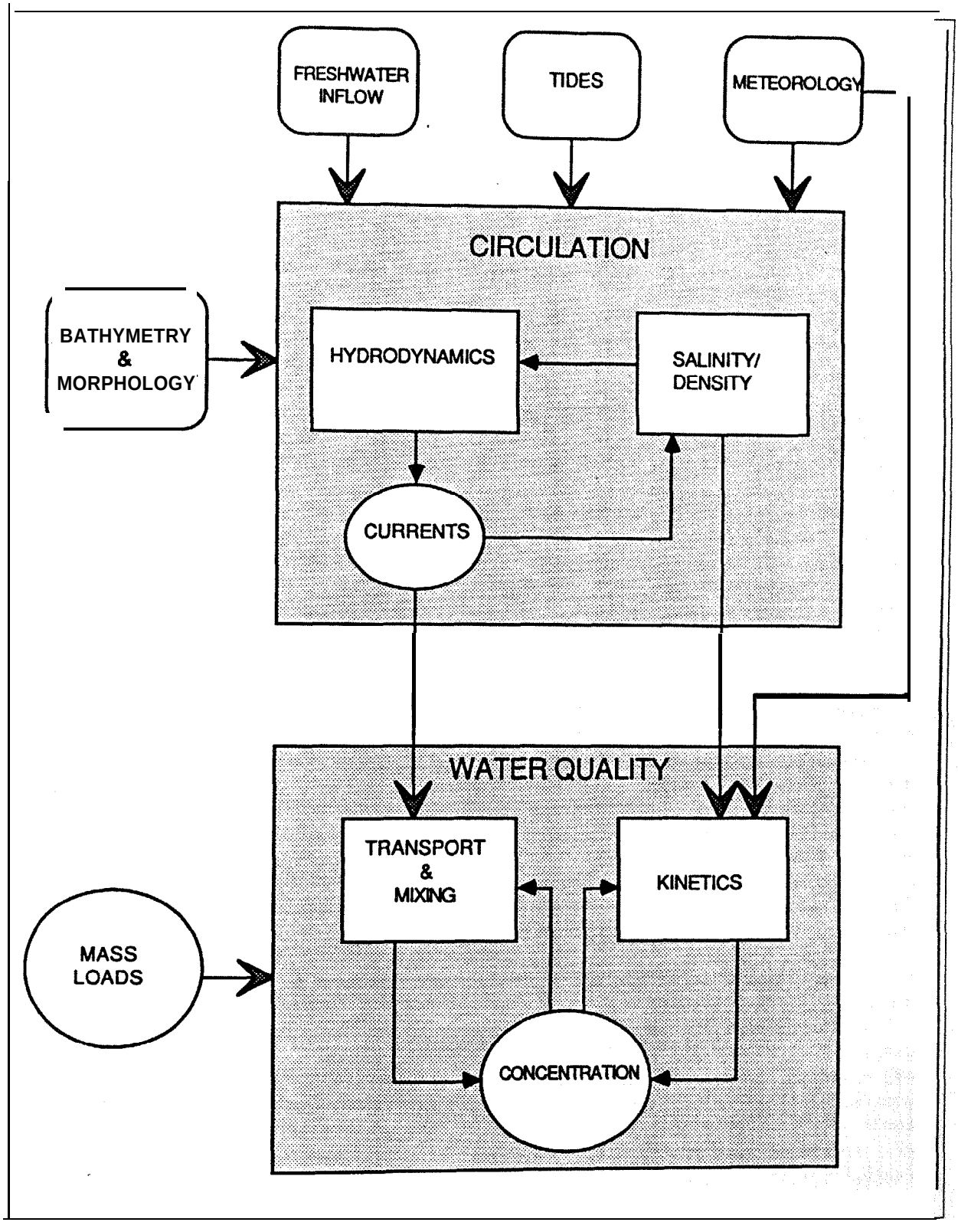

Fig. I. Principal controls on estuary water quality.

ment is: for the specific estuary problem of concern, how can the model simplify this complex reality and still depict the constituent concentrations to an adequate accuracy? Model formulation must be based upon a careful analysis of the management problem, identifying the space-time scales of importance, and the factors controlling the estuary response at those scales. Because any model is a simplification of nature based upon various assumptions, it is necessary to check that the model achieves its intended Purpose by comparing its "predictions" with actual measured data. This is the process of model verification (e.g.. Thomann and Barnwell 1980).

In the Present context of aquaculture development, we address a specific management problem-the determination of the estuary carrying capacity for aquaculture. This is not a new Problem in itself. Estuaries that receive high loads of wastes are frequently subjected to an analysis of assimilative capacity. In the United States, this has been carried one step further---to form the basis for so-called wasteload allocations (Southerland *et al.* 1984), in which specific wasteload limits are imposed on individual dischargers to maintain a lower bound on water quality throughout the receiving watercourse. The general procedure of assimilative capacity &termination is given in Fig. 2. The Process starts with determinations of:

- critical conditions, i.e., that combination of external controls that maximizes impacts of the wasteload on water quality—for example, law river flow and high temperatures;
- the concentration level that determines acceptable water quality ("threshold of impact" in Fig. 2).

A suitable mathematical model is used to determine the concentration that results from a given level of wasteload. This model is indicated by the shaded boxes of Fig. 2, emphasizing that for an estuary there is both a hydrodynamic and a mass balance aspect of the modeling. The wasteload magnitudes are then adjusted until the predicted concentration is equal to the threshold of impact. This wasteload value is the greatest that can be discharged without exceeding the specified threshold, and is, therefore, the assimilative capacity for the system.

This is the basic Procedure needed to determine the carrying capacity of an estuary for aquacukure. The specific concern may be the wasteload from the operation, in which case the procedure of Fig. 2 applies directly. It may also be the effects of other aspects of the operation, such as diversion and release of Pond Fig. 2 waters, or physical modification to the estuary to accommodate aquaculture, in which case the "feedback loop" of Fig. 2 leads back to the hydrodynamic part of the computation, rather titan the wasteload. Or, of course, all of these may be involved.

For simplicity the carrying capacity analysis procedure is Presented as though it would be applied to the estuary *in toto*. In fact, the assimilative capacity determination is a strong function of position in the estuary. There will be areas in almost any estuary that will generally have a high degree of assimilative capacity, and are well-circulated and subject to regular water mass replacement. There will also be areas that are poorly circulated with frequent stagnation (dead zones), which will have a low assimilative capacity. The location of the aquacuhure operation relative to we&circulated or poorly circulated zones, and relative to existing wasteloads, is important to the ability of the estuary to assimilate its wasteload or respond to its circulation modifications.

These considerations of wasteload position and regional circulation characteristics in the estuary also determine the appropriate time and space scales of analysis. The unit of measure is the tidal excursion. If the zone of degraded water quality is located within a tidal excursion of the point of operation (a wasteload), then the model time resolution must be intratidal, and capable of detailed spatial resolution, at least in the vicinity of the operation. On the other hand, if the zone of degraded water quality is distant from the region of the operation by several or many tidal excursions, then an intertidal, or long-term average analysis will probably be sufficient, with only large-scale spatial depiction.

Such a carrying capacity analysis requires a considerable amount of preparatory work before the procedure of Fig. 2 can actually be carried out, The following nontrivial tasks must have been performed: (1) specification of which water quality parameter(s) will form the basis of water quality evaluation; (2) definition of the parameter value(s) corresponding to acceptable water quality; (3) development and verification of a model for the specified parameter(s) that is appropriate for the estuary of concern; and (4) determination of the combination of external conditions that are critical for water quality.

The definition of "acceptable water quality" in (2) may be based upon maintenance of certain biological communities in the area-for example, a minimum level of dissolved oxygen for the estuarine fishery. In some situations, the aquaculture operation may itself require a minimum standard of quality in the estuarine waters for influent pur-Poses. If this standard is controlling for the carrying-capacity analysis (that is, is most stringent of all of the applicable water quality standards), and the aquaculture operation itself affects the constituent concentration involved, then there is the possibility that aquaculture can be self-limiting in that estuary. Development beyond the carrying capacity level can render aquaculture nonviable in the system.

Frequent reference is made to the "flushing time" of an estuary, especially in the aquaculture community. This is defined as T = V/Q where T is the flushing time, V is the volume of the estuary and Q the long-term average river inflow (e.g., Zimmerman 1971, Officer 1976). This is a

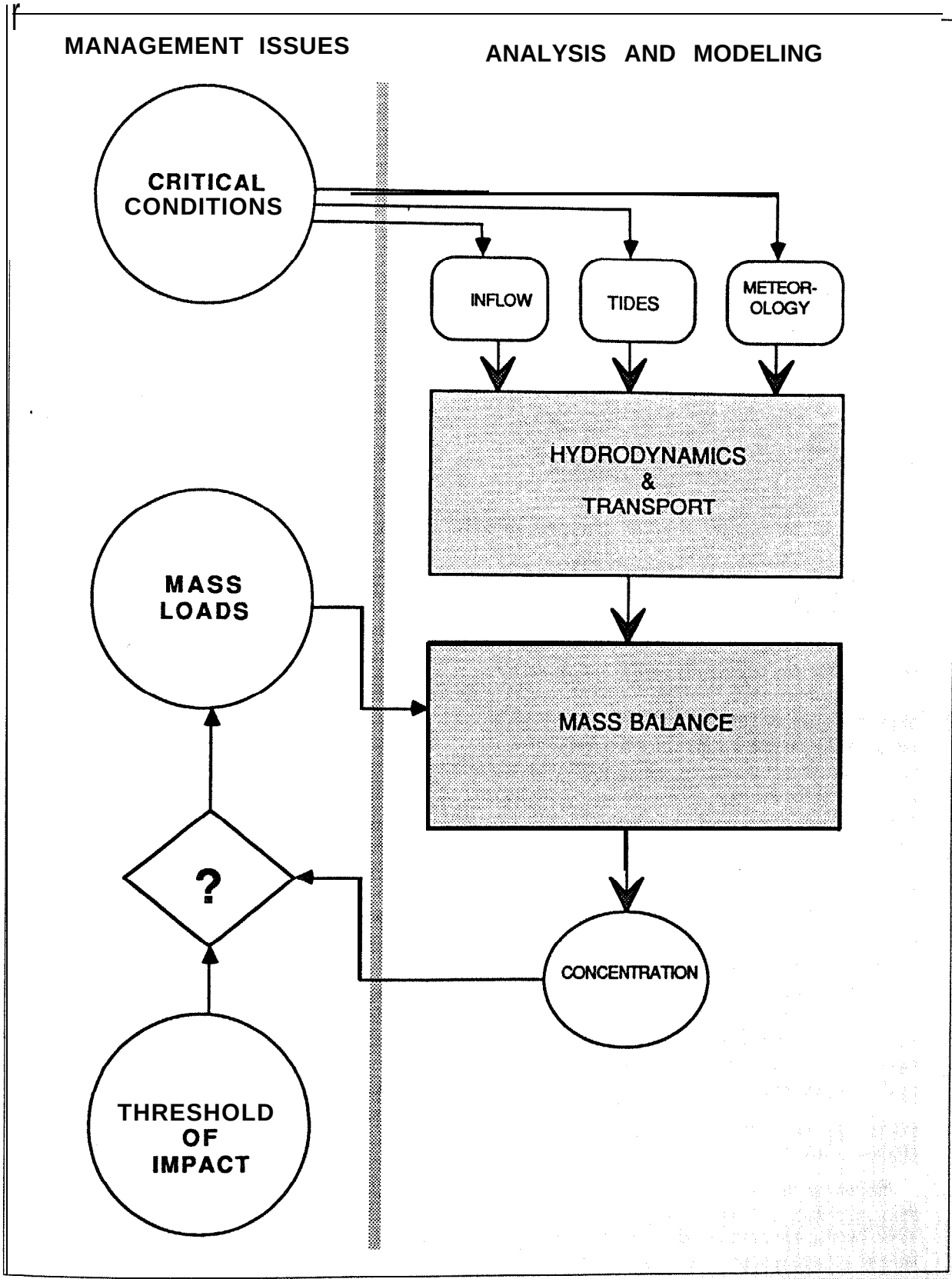

Fig. 2. General method for determination of assimilative capacity.

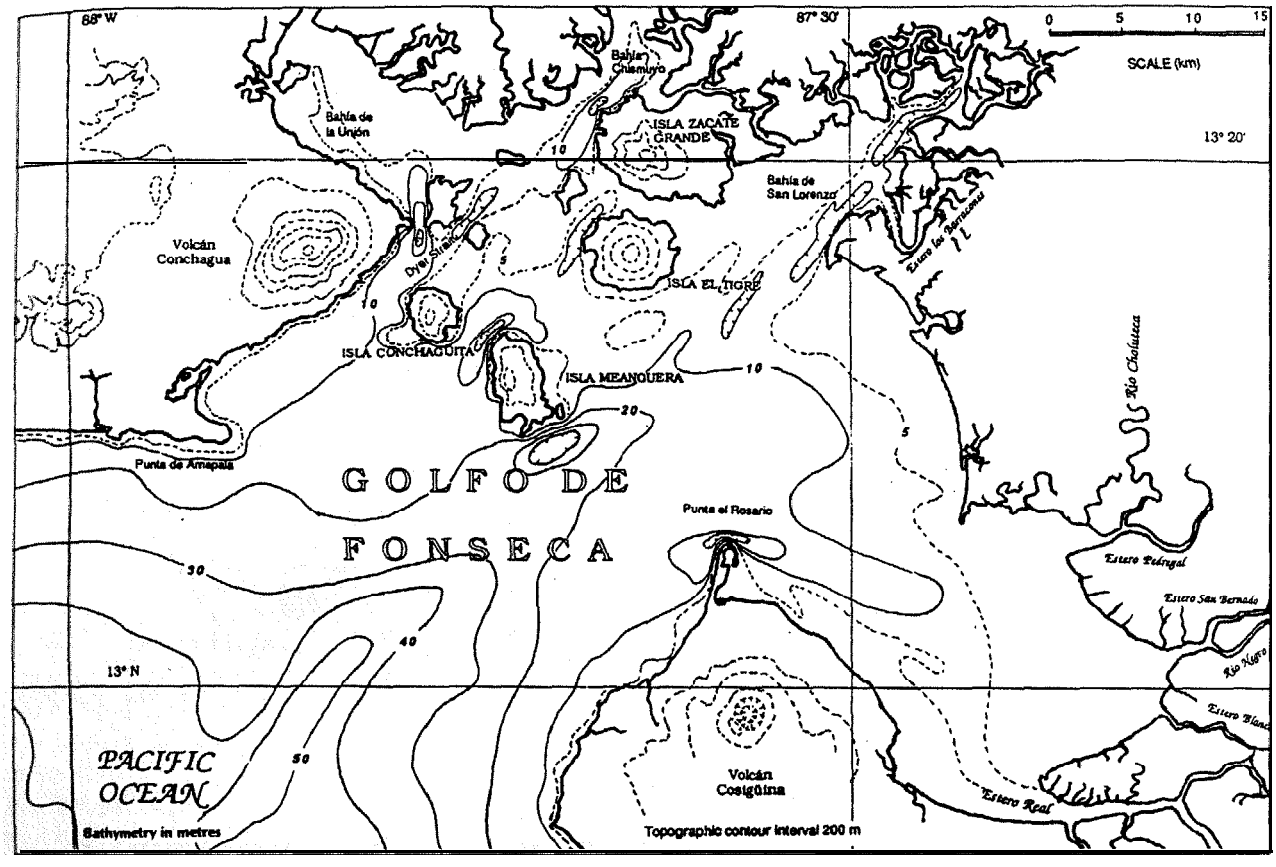

Fig. 3. Golfo de Fonseca: general morphology and bathymetry.

concept that has been imported into the estuary from lakes and rivers, Also referred to as "renewal time" and "replacement time," this is the time required for the freshwater inflow to replace the volume of water in the estuary. It is directly related to the degree of dilution with "new, uncontaminated" water. In au estuary, the parameter is nearly useless, for two reasons. First, dilution varies strongly as a function of position in the estuary. A single number attempting to characterize the entire system is useful only for gross, relative comparisons between estuaries, not for any absolute characterization of the estuary's ability to assimilate wasteloads. Second, there are other mechanisms of dilution and water replacement operating in an estuary in addition to river inflow. More importantly, there are tides, meteorological flushing and the influx of seawater driven by density currents.

CASE STUDY: SHRIMP AQUACULTURE IN HONDURAS

Shrimp farming has been conducted for 25 yr in Honduras, starting with the experimental farm of Sea Farms in 1970, but has been commercially viable only for the last decade. Shrimp is now the third largest export of Honduras* after bananas and coffee. The shrimp farming industry in Honduras is concentrated around the Gulf of Fonseca, a large estuary on the Pacific Coast, volume about 1.7 x 10^{10} m^3, that comprises the common boundary of El Salvador, Honduras and Nicaragua. The dominant species are the Pacific white *(Penueus vannamei)* and the Pacific blue (*Penaeus stylirostris*), both of which are native to the area. The critical element in the development of the commercial industry was the discovery that sufficient wild postlarvae could be harvested from the tidal flats to support seeding of the ponds.

The morphology and bathymetry of Golfo de Fonseca are shown in Fig. 3. This estuary as a whole is a tectonobay, but its inland reaches exhibit features of a drowned-river-valley-type estuary, with extensive mud shoals, and deltaic-like shoal areas, especially its eastern arms. Its coastal physiography consists of tidal flats, tidally-flushed mangrove swamps fringing largely unvegetated tidal flats, and low-relief "sweetland" punctuated by steep igneous formations.

The tide is semidiurnal. dominated by the phase of the moon (i.e., the spring neap cycle) and ranges 1 m (neap equatorial) to 4 m (spring tropical) in the open bay. Several deep, tidally-scoured channels are evident in the bathymetry. The coastal pilot directions include numerous warnings about strong currents. An example of the interior tide is shown in Fig. 4, from a temporary water-

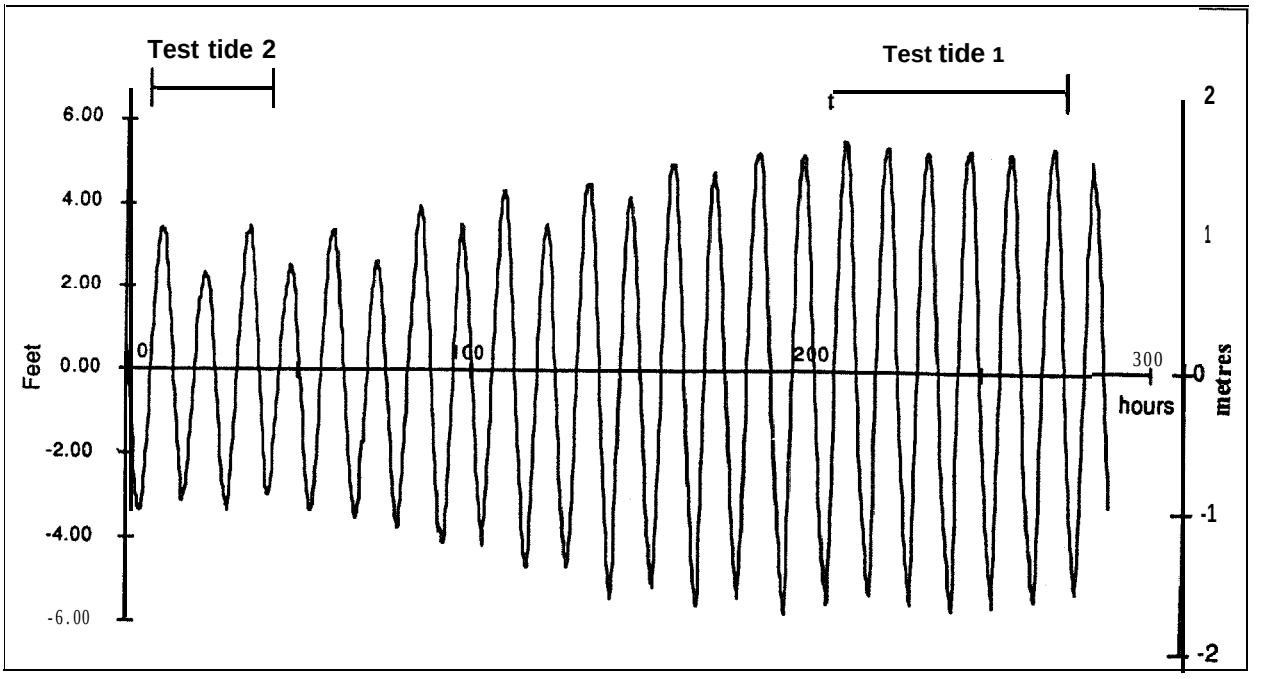

Fig. 4. Observed tide 1-12 August 1994 in Estero el Pedregal, Granjas Marina, intake.

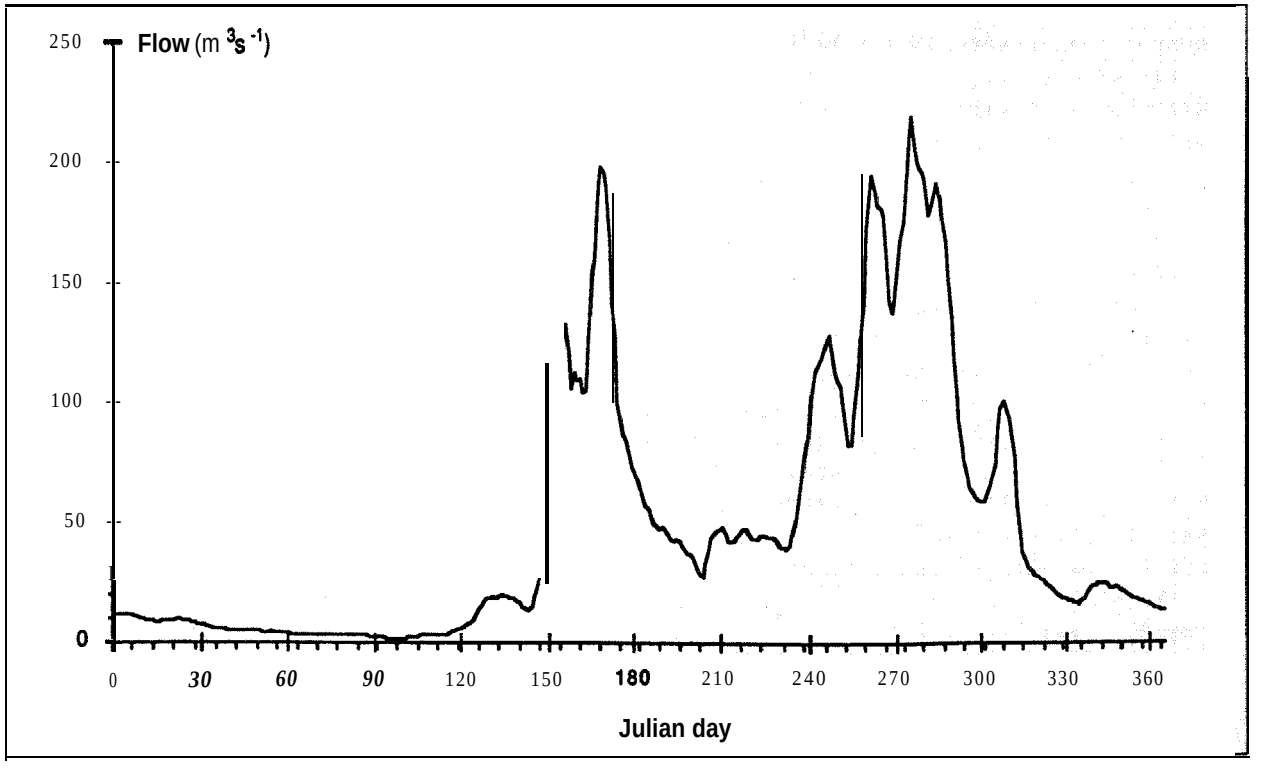

Fig. 5. Average 1979-1990 daily flows,7-day running mean, Rio Choluteca.

level installation at the Granjas Marinas intake on Estero et Pedregal on the eastern shore (D. Teichert-Coddington, Department of Fisheries and Aquaculture, Auburn University, Auburn, AL, pers. commun. 1995).

Several rivers flow into Fonseca, the most important of which is the Rio Choluteca, which drains most of Honduras west of the continental divide. Precipitation in this area of Central America is driven by intense local thunderstorms embedded in tropical depressions. Numerous dendritic drainageways flow into the side bays from peripheral runoff. The seasonal behavior of flow in the Rio Choluteca is shown in Fig. 5, which displays the 1979-90 average for each day, further smoothed by a seven-day running mean to filter out the hydrographic peaks (based upon daily measurements of the Puente Choluteca gauge provided by the Departmento de Servicios Hidrologicos y Climatologicos). Runoff is clearly bimodal, with two high-flow seasons, spring and fall, separated by the dry seasons of winter and summer. The winter dry season typically extends from November through May, during which season the region becomes quite arid, exacerbated by high evaporation rates due to high temperatures, high winds and reduced humidities. The river flow regime during the dry season becomes five months of virtually steady flow an the order of 5- 10 m^3/s. The "little dry season" of summer, which typically occurs in July-August, is usually only a two-month interruption of the thunderstorm season. It is reasonable to assume that the gauged flow of the Choluteca accounts for half of the inflow to the estuary, which would imply a total mean annual flow on the order of 100 m^3/s.

The Rio Choluteca also drains the urban areas of Tegucigalpa and Choluteca in Honduras and receives the wastewater from both of these municipal areas, about 25% of the population of the country. Assuming a combined population in the watershed of 1 million, with a per capita oxygen demand (BOD) of 0.1 kg/day (0.25 lb/day), the total load would be on the order of 100,000 kg/day (250,000 lb/day). Data from the river downstream from Choluteca (and above tidal influence) show relatively low values of BOD, but elevated concentrations of inorganic nitrogen (- 0.5 ppm) and filterable phosphate (~ 0.25 ppm), which suggest that most Of this wasteload is stabilized in its transit down the river channel (D. Teichert-Coddington, Auburn University, Auburn, AL. pers. commun. 1995). It is probable (though no data are yet available to confirm this) that the gauged flows in the dry season are predominantly wastewater return flows.

The shrimp farming industry has become concerned about water quality problems that could occur in association with shrimp aquaculture on Golfo de Fonseca, especially whether shrimp farming could become self-limiting by degrading the water used for pond exchange. Specific concerns include reduced oxygen, excessive nutrients, pathogens and toxins, high suspended solids, and elevated salinities in the influent water.

While the magnitude and geographical distribution of the mass influxes of contaminants are clearly an important control, an equally important control is the hydrodynamic capacity of the estuary for dilution and transport. In other words, the hydrography of the estuary dictates the relation between mass loads of contaminants and the severity of the resulting water quality. An action which alters either the hydrography or the wasteloading has the potential of altering water quality. Shrimp farming can do either.

As a quantitative demonstration of this, as well as a demonstration of how estuary modeling can be employed in management decisions concerning aquaculture development, we consider a single subestuary of the Gulf of Fonseca, Estero el Pedregal, a river-channel estuary in the southeastern arm of the system. The Pedregal is selected because (1) it is a system with relatively simple geometry, allowing application of one-dimensional models, (2) it receives the inflow of Rio Choluteca, so we have good information on gauged river flows, (3) it is the site of some rather concentrated shrimp farming operations, and (4) a good data base on physicochemical data has been collected over the past two yr by a cooperative program between the shrimp farmers, federal agencies and universities (D. Teichert-Coddington, Auburn University, Auburn, AL., pers. commun. 1995).

The Pedregal is one distributary of a large fluvial swamp/marsh complex in the eastern segment of Golfo de Fonseca (see Fig. 3). It is a network of dendritic channels maintained by tides and seasonal runoff, which incise extensive tidal flats. The tidal channels are fringed by dense growths of mangroves. There are two main tidal channels in the Pedregal, the Estero el Pedregal per se, and the Estero la Jagua, which receive the inflow from the Rio Choluteca and conflows with the Pedregal 2 km upstream from its mouth (Fig. 6). An important geometric feature of the Pedregal is its sharply declining cross-sectional area with distance upstream: it is a horn-shaped estuary, whose cross-section drops from nearly 25,000 m^2 at its mouth to less than 50 m^2 in about 30 km. Therefore, the channel itself has a quickly diminishing capacity for flow, as well as a quickly increasing resistance to flow. An equally important feature is the large tidal flats which communicate with the main tidal channel through small scoured tidal passes through the mangrove fringe. These tidal flats have the capacity to store a great amount of water on the rising tide and to release that water back into the tidal channel as the tide stage falls.

The general locations of the shrimp farming concessions in late 1993 are indicated in Fig. 6; however, these do not necessarily correspond to the boundaries of the shrimp ponds. Data on actual producing-pond areas as of 1994 for the larger operations are given in Table 1. These shrimp farms eliminate the tidal flats, hydraulically iso-

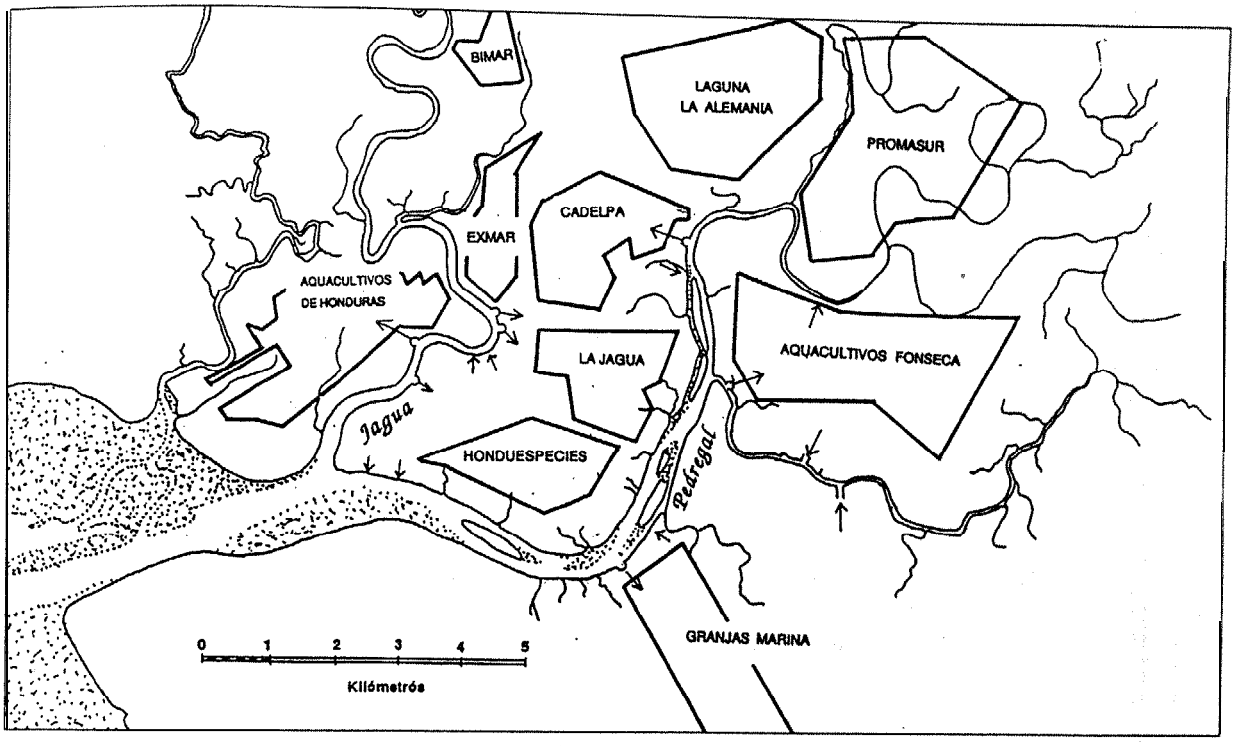

Fig. 6. Estero el Pedregal region, showing present shrimp farm concessions.

lating these areas by enclosure within levees to create shrimp ponds.

A tidal hydrodynamic model of Hauck and Ward (1980) was applied to the combined Pedregal-Jagua system. For simplicity, only these two channels were considered-the main channel of the Pedregal and the main channel of the Jagua. This model is a numerical solution to the differential equations of momentum and continuity and provides a means to compute tidal currents in the estuary based upon the measured tidal stage. Time integrations of several tidal cycles were carried out, solving for tidal current and water level throughout the estuary, from which three key hydrodynamic indicators were determined:

- tidal excursion: the distance that a parcel of water moves on the flooding tide,
- mean tidal-current speed: useful in estimating dispersion and reaeration, and
- tidal prism: the volume of water carded past a fixed point on the flooding tide.

Two scenarios were examined: (1) the pre-aquaculture geometry, with flooding tidal flats, as indicated on topographic maps of this region and (2) 1994 shrimp farm development, in which the tidal-flat areas were reduced by the amount of production areas shown in Table 1. A striking difference in tidal prism between the natural geometry and the shrimp farm development was found. The elimination of 1500 ha of tidal flats along the Pedregal reduces the tidal prism in the lower reaches of the estuary by 10-35%, and the elimination of 1010 ha along the Jagua reduces its

Table 1. Shrimp farm pond acreage used in hydrodynamic modeling experiments for Estero el Pedregal and La Jagua

Farm	Total pond ares (ha)* (ha)	Estuary tidal flats on Pedregal (ha)	on Jagua (ha)
Granjas Marinas S.B.	1850	1000	
Cadelpa	360	180	180
Aqua. Fonseca	960	70	
La Jagua	100	50	50
Honduespesies	400	200	
Aquacultivos Hond.	580		580
Honduespecies	400		200

[a] Data from SAPROF Team (1992) and Teichert-Coddington (pers. commun. 1994)

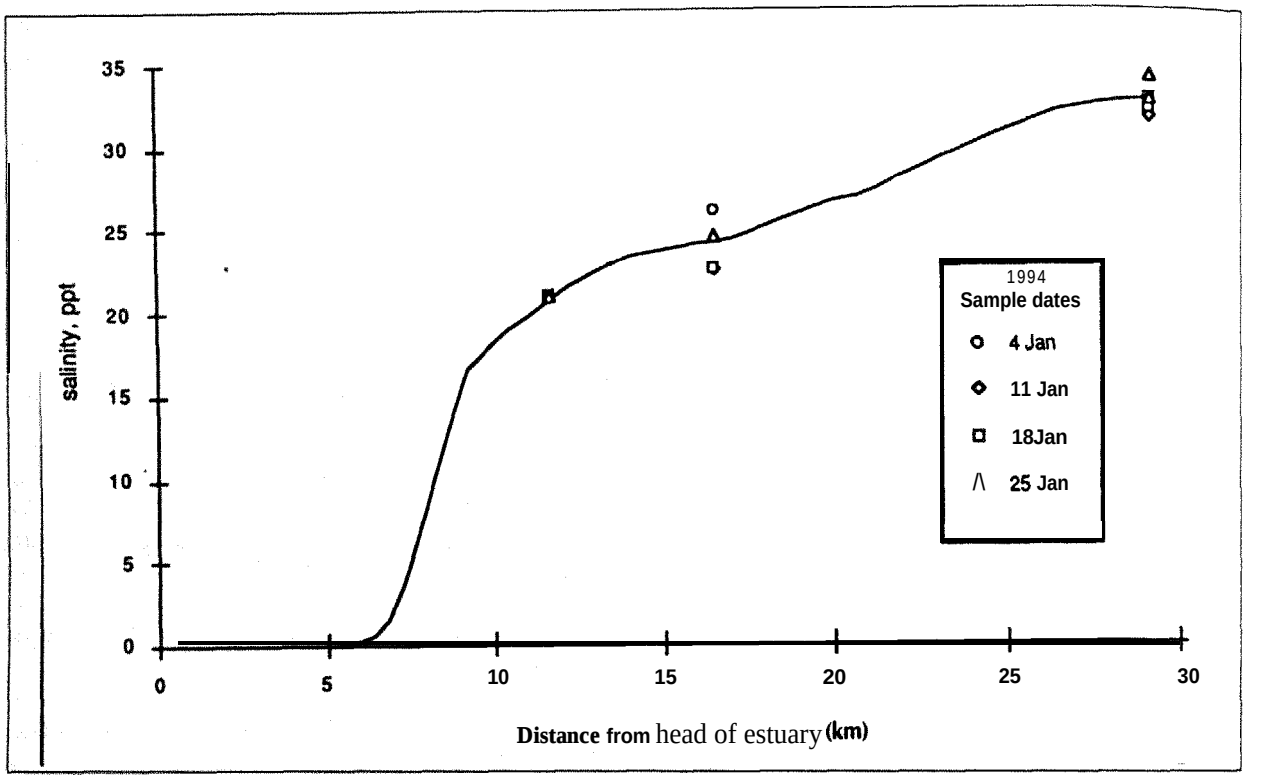

Fig. 7. Estero el Pedregal salinities, moderate flow: model simulation and observations.

tidal prism in the lower reaches by nearly 25% The reason is clear: the removal of this area reduces the capacity of the estuary to store water on the rising tide, so the amount of water entering the estuary is diminished proportionately. This translates to a direct reduction in the diluting capacity of the estuary's tidal exchange.

The distribution of various constituents in the estuary is the central concern in determining assimilative capacity and the potential for self-limitation. In this case study, dissolved oxygen (DO) was examined As one of the most fundamental measures of estuary quality, it is certainly an important constraint on suitability of estuary water as shrimp pond influent. Its concentration was determined by application of a mass transport model, using the same numerical segmentation as the tidal hydrodynamic model. A long time scale was appropriate, so a tidal-averaged steady-state model was employed. Two different levels of river flow were examined, one corresponding to the dry season base flow, the other to a moderate level of inflow that still allowed some salinity intrusion into the Pedregal.

Both salinity and dissolved oxygen were modeled. Although salinity in the estuary is not really susceptible to management control, modeling of salinity nonetheless serves several important functions. First, because salinity is a natural conservative tracer, it can be used to verify the ability of the model to compute advective and dispersive transport, by comparison of the model results to salinity data Second, salinity exerts a control on some of the kinetic processes affecting other parameters; for example, oxygen saturation is reduced with increasing salinity. Third, the location of the horizontal salinity gradient can be an indicator for other processes potentially important to shrimp farming. One important example is the accumulation of fine sediments in the region of the salinity gradient caused by a convergence of sediment carried by the density current circulation. The model prediction of salinity for the higher flow regime (January 1994) in the Pedregal is shown in Fig. 7, along with salinity observations at the intake sites for several of the farms.

In order to model dissolved oxygen, biochemical oxygen demand (BOD) must be modeled first and fed-forward into the dissolved oxygen calculation. This requires inputs on the oxygen-demanding wasteloads, which were assumed to be the Rio Choluteca load and the effluents from shrimp ponds. The latter are tabulated in Table 2. This also requires information about the sources and sinks of both BOD and DO, of which we have practically no information in this system. For the purposes of this exercise, some order-of-magnitude judgements were made. AS matters turned out, under the higher flow regime, the BOD and DO of Estero la Jagua are dominated by the quality of the Rio Choluteca inflow and theshrimp farms have only

Table 2. Shrimp farm BOD toads used in dissolved oxygen modeling experiments for Estero el Pedregal and La Jagua

Farm	Present		Projected	
	pond area[a] (ha)	BOD load (kg/d)	area (ha)	BOD load (kg/d)
	Drainage to Pedregal			
Granjas Marinas S.B.	1850	23000	3000	46000
Cadelpa	360	9000	500	11000
Aquacultore Fonseca	960	18000	1000	23000
La Jagua[b]	100	1100	200	2300
Honduespecies[b]	400	4000	500	6000
Alemania	400	4500		
Promasur	400	4500		
TOTAL Pedregal	3670	55100	5000	97300
	Drainage to Jagua			
Aquacultivos Hond.	580	11000	700	16000
Honduespecies[b]	400	4000	500	6000
La Jaguab	100	1100	200	2300
BIMAR	100	5000	100	5000
EXMAR			200	9000
TOTAL Jagua	1180	21100	1700	38300
GRAND TOTAL	4850	76200	6700	135600

[a] Data from SAPROF Team (1992) and Teichert-Coddington (pers. commun. 1994)
[b] Assumed to drain equally to both estuaries

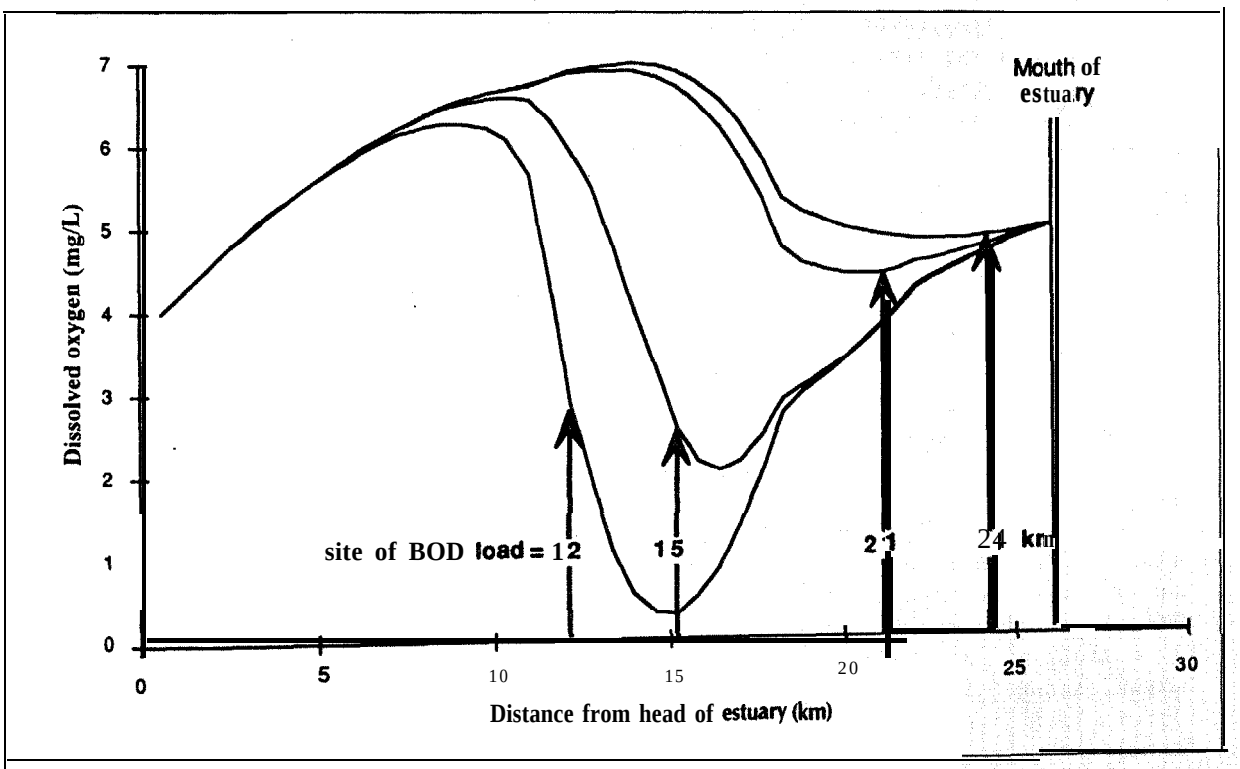

Fig. 8. Dissolved oxygen profile in Estero la Jagua resulting from single 11,000 kg/day BOD bad.

minor influence, which is consistent with the findings of Teichert-Coddington (pers. commun. 1994) based upon the chemical data he has collected along the estuary. The greatest impact of the shrimp farm operations on estuary quality occurs, rather, for the dry season flow.

Once a model is available, it allows insight into important features of an estuary by "what-it" scenarios. As an example, we consider only a single shrimp farm operating on the Estero la Jagua, namely the Aquacultivos Honduras, under dry-season conditions. This farm drains into the Jagua about 1.5 km from its confluence with the Pedregal. Here the estuary channel is wide and deep; there is a large tidal prism and free exchange with the waters of the Gulf of Fonseca, so the effect of the effluent on BOD or DO in the receiving water is negligible, as shown by the "24-km" model curves in Fig. 8. Using the model, we move the shrimp farm to points farther upstream, namely 4, 10 and 13 km, corresponding respectively to 21, 15 and 12 km points (measured from the head of the estuary) in Fig. 8. The farther upstream one goes, the smaller the estuary cross-section, the smaller the tidal prism and the poorer the exchange (dispersion), so the same BOD mass load results in a higher BOD concentration. The result is a greater impact on the dissolved oxygen. For any point farther upstream than 13 km, the DO is driven to zero by this one shrimp farm. Of course, even with the farm at this location, the oxygen is too low to sustain aquatic life: the 15 km position (10 km from the mouth) would probably represent the lowest oxygens that could reasonably be tolerated by estuarine organisms. This experiment illustrates that the impact of a specific shrimp farm depends not only upon the effluent load of that farm but also where it is located within the estuary. This experiment also illustrates that a mass load in such a highly dispersive system as these river-channel estuaries affects quality a great distance both downstream and upstream from the point of discharge.

Model simulations of dry season DO in the Pedregal and Jagua systems under the present shrimp farm development are shown as the thin curves in Figs. 9 and 10. A future development scenario was projected based upon assumptions of expanded pond area that could be feasible under the existing Honduran concessions. These are hypothetical only, but represent a not-unrealistic picture of how shrimp farming might expand in this area in the foreseeable future. The bold curves in Figs. 9 and 10 depict model predictions of DO under the projected BOD loading, all other factors remaining constant. What level of DO should be taken as critical has not been established for these systems, but a value of 3 mg/L for aquatic life, including shrimp pond influent, would be reasonable. In this case, the present level of shrimp aquacuiture in the Pedregal would appear not to be excessive, but in the Jagua would be marginally stressed below the critical value in the lower estuary. Under the projected development scenario, both the Pedregal and the Jagua show DOs reduced substantially below this critical value; in the case of the Jagua there is an anoxic reach of several kms. These results assume constant geometry. If the dispersion is reduced to account for the proportional reduction in tidal prism, the low DOs in the Pedregal are driven down to values on the order of 0.5 ppm. Clearly-assuming that the model parameters are correctly quantified-the assimilative capacity of both systems would have been exceeded at the projected level of development.

Actual measurements of dissolved oxygen would be extremely valuable in assessing the model performance, Unfortunately, very little of this kind of data is available from Honduras. The few DO profiles that have been performed in either the Jagua or Pedregal were taken under less critical conditions than those modeled above; however, DO sags were observed in the vicinity of the pond drains. There is indirect evidence of degrading water quality in the shrimp farming areas. Intake records of Granjas Marinas from its intakes on the Pedregal show frequent episodes of low DO especially during the dry season. Several large fish kills have occurred in the esteritos, and there has been a reported decline in the artisanal fishery of all species. More telling, perhaps, is the sharp decline in catch and catch-per-unit-effort of wild postlarvae (PL) in recent years, which has now necessitated the Honduran industry to seek other sources for PLs, including importing of third-party PLs and construction of hatcheries. It should be noted that the channel estuaries, like Jagua and Pedregal, are the main avenues for migration of PLs into the tidal flats and mangrove swamps upstream. DO sags in these estuaries would effectively block this migration and deny access of the PLs to the upstream nurseries.

CONCLUSIONS

Two principal conclusions about shrimp farming on the Gulf of Fonseca, which may apply more generally to other similar aquaculture industries on estuarine systems, follow from this analysis. The first is that the hydrographic conditions in the regions in which shrimp farming operates are demonstrated to be at least as important as chemical quality in determining the suitability of the water for influent supply. This will probably prove to be the case for other aquaculture Operations on other estuaries. These hydrographic conditions include:

- tidal range and period,
- freshwater throughflow,
- physiography and morphology-especially the role of tidal flats,
- tidal currents and parameters determined from the Currents-such as excursion and prism,
- mixing and dispersion,
- salinity-especially gradients of salinity.

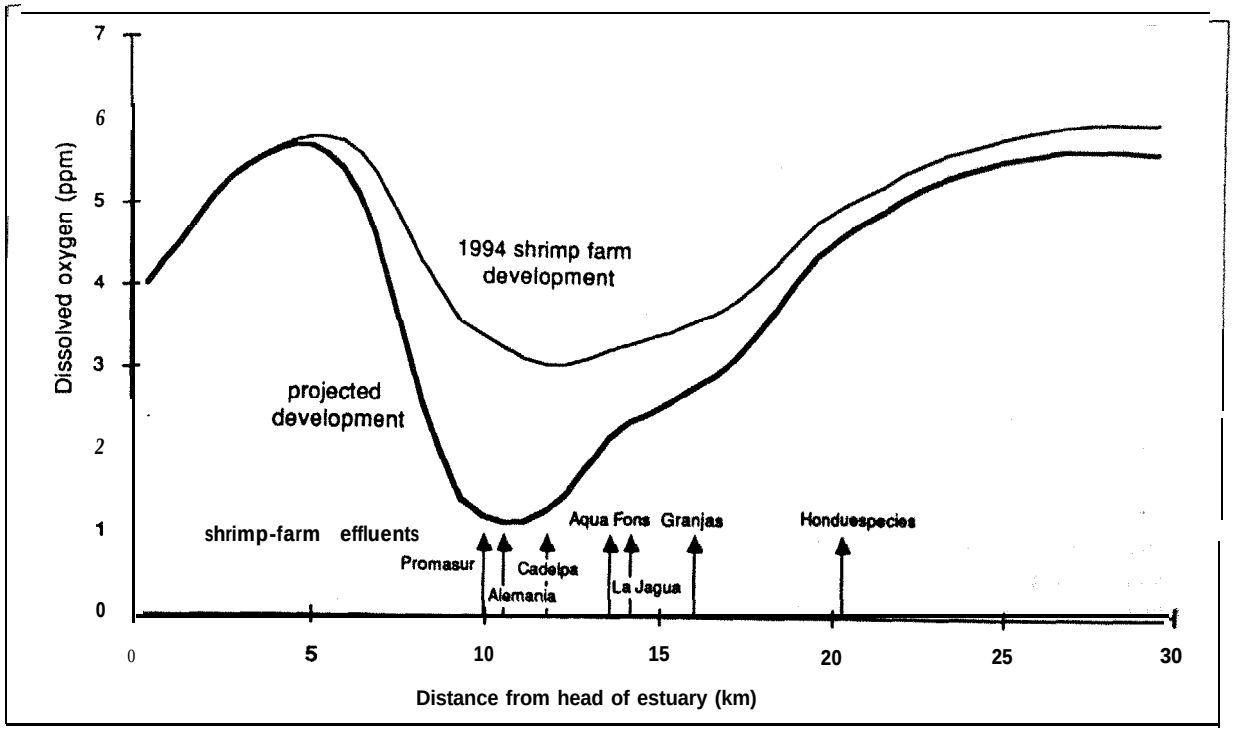

Fig. 9. Estero el Pedregal dissolved oxygen profile for two scenarios of shrimp farm development.

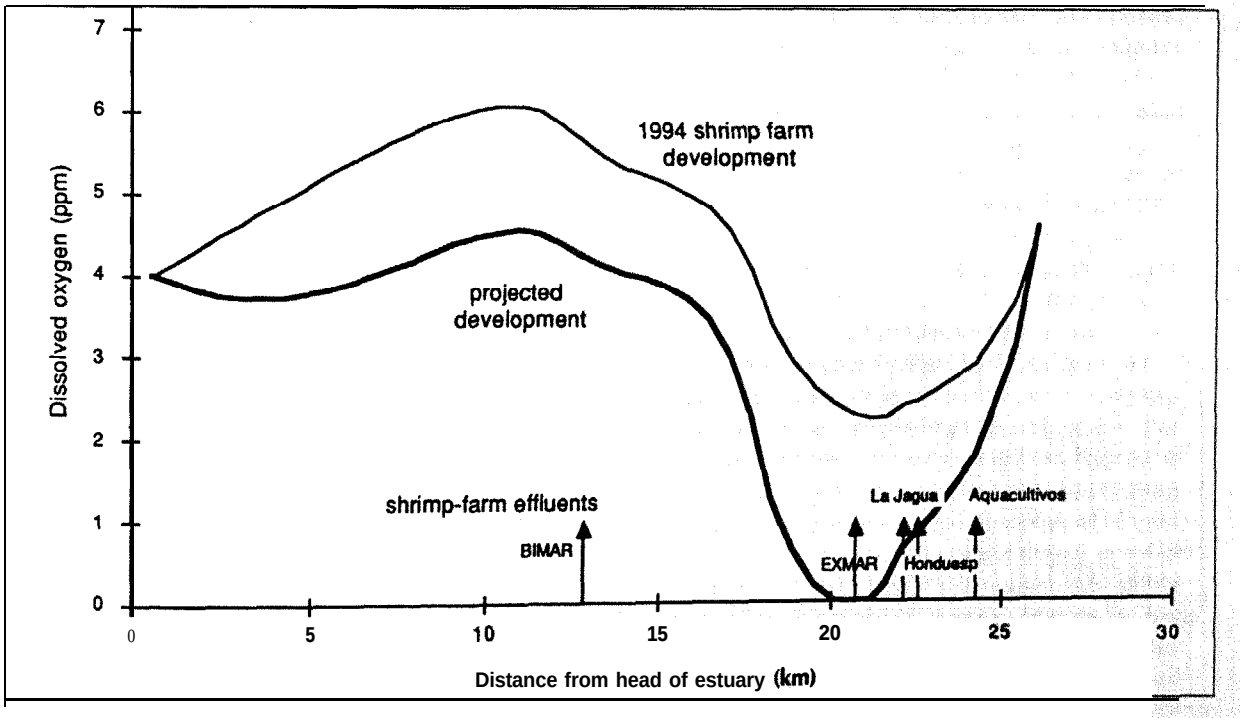

Fig. 10. Estero la Jagua dissolved oxygen profile for two scenarios of shrimp farm development.

The second conclusion is that for systems such as Estero el Pedregal and Estero la Jagua, which are typical of many of the river-channel estuaries within the larger Gulf of Fonseca along which shrimp farming is being developed, there is a level of development at which the estuary becomes so degraded as to prohibit economical aquaculture, i.e., shrimp farming becomes self-limiting.

In this case study, we demonstrated this self-limiting effect by application of two rather simplified models, concentrating only upon dissolved oxygen. The same kind of modeling could be applied, with some minor modifications, to nitrogen and phosphorus nutrients, and to specific toxicants such as ammonia or indicators such as chlorophyll a. Also, the resolution of the tributaries can easily be increased, with multiple channels and extending the model to the heads of the tide. Such a model can be used to better define critical conditions and to evaluate any number of different shrimp farm development scenarios, to see which would be possible given the hydrographic environment, and which would result in unacceptable degraded water quality. There are other kinds of operational problems for which this type of model would not be appropriate, but for which others would. Some of the deeper, more energetic subestuaries in the Gulf may require more complex models, perhaps including the vertical dimension. Also, there are smaller scale problems which could be addressed using intratidal models. One of the most important is the entrainment of effluent into the intake of a farm, either from drainage from other farms or from the same farm. In short, the technique of modeling offers a great capability for management of shrimp farming and its development in an estuary,

One of the greatest limitations to this approach is the information base needed to carry out the necessary modeling, Foe Honduras, the necessary data is sorely lacking. In order to produce the model results of this case study, we had to make numerous assumptions. While these were educated guesses and are considered to be at least qualitatively correct, much more accurate information is needed to be Confident of the model results and before modeling Can be used in the management process.

The problem of an inadequate dam base is endemic in estuaries and will probably be the situation for any region with potential for aquaculture. Because of the wide range in external conditions, seasonal variation and many time-space scales of variability, a data collection program will have to be sustained for a considerable period of time in order to permit comprehensive analysis of estuary response Acquisition of a suitable data base is generally the task of greatest urgency in implementing a strategy for carrying capacity analysis. The foundation of data collection should be measurements of tides, salinities and water chemistry within the immediate regions of the existing and proposed aquaculture operations, and throughout the estuary itself. The expense of operating boats, and the need for accumulating data from a series of surveys over a period of time imply a significant investment. However, this investment is miniscule in comparison to the capital investment in aquaculture facilities. Moreover, the potential return on this investment, in ensuring the continued economic viability of aquaculture in an estuarine setting, is huge.

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Effects of Water Movement on the Fluctuation of Oxygen Concentration in the Lower Layer of Gokasho Bay on the East Coast of Honshu Island, Japan

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ABSTRACT

The physical factors affecting the fluctuations of oxygen-deficient water masses in the lower layer (below the pycnocline) of Gokasho Bay were investigated. In this area. sea bream, yellowtail and pearl oysters are cultivated. An oxygen budget was estimated using a two-layered box model analysis with the results of field observations. The effects of water movements on the fluctuation of dissolved oxygen (DO) concentration were then examined. Oxygen-deficient water masses existed in the lower layer throughout the stratified season (from May to October). The factors which caused the oxygen depletion were clarified by the results of an oxygen budget analysis. In summer, oxygen depletion was caused by low vertical diffusion. In early autumn, a very high consumption rate of DO caused oxygen depletion, despite high vertical diffusion and high horizontal advection. Water masses from outside the bay were found to intrude into the bay intermittently. DO concentrations showed short periodic fluctuations due to these intrusions. The intrusions were caused by coastal upwelling and concomitant ascent of the pycnocline outside the bay. Because DO concentrations outside the bay were. higher than those in the lower layer of the bay during the stratified season, the intrusions increased the supply of DO in the bay. The oxygen-deficient water mass disappeared temporarily after the intrusion. Water movement was the significant factor which supplied DO in the bay during the stratified season.

INTRODUCTION

Many workers have described the spatial and temporal distribution of the oxygen-deficient water masses in the coastal area of Japan (e.g., Ochi and Takeoka 1986, Joh 1989, Sasaki 1989). To clarify the processes of oxygen depletion, it is important to study the physical, chemical and biological factors that control water quality in the bay, Munekage et *al.* (1991) observed the effects of the intrusion of oceanic water on oxygen depletion and Unoki *et al.* (1985) considered water movements in his study of oxygen consumption rates. Water movements and their effects on dissolved oxygen (DO) concentrations differ among the bays; the physical characteristics of each bay must therefore be examined to elucidate the mechanism of oxygen depletion. In this paper, we examined the physical factors affecting oxygen depletion in Gokasho Bay. Coastal water masses outside the bay intrude into the bay intermittently (Abo et *al.* 1996). We conclude that such intermittent events of water movements are important to the fluctuations of oxygen-deficient water masses.

OBSERVATIONS

Gokasho Bay is located on the southern coast Of central Japan (Fig. 1). Sea bream, yellowtail and pearl oysters are cultivated in this bay, and fish farms are concentrated in one of the inlets of the bay (stn. 6-8).

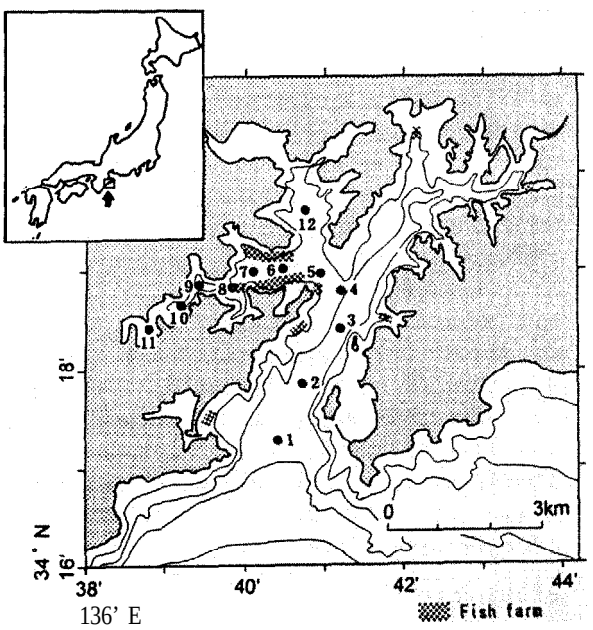

Fig. 1. Map of Gokasho Bay and the observation stations. Heavily stippled areas denote the distribution of fish farms

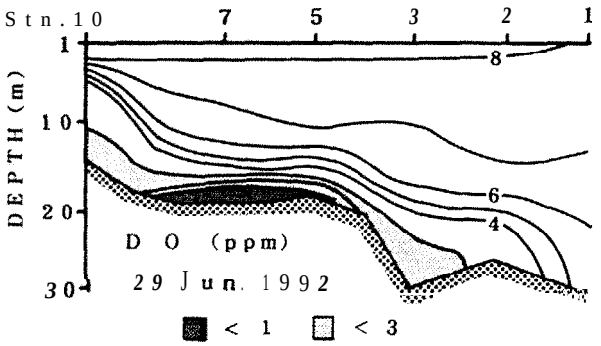

Fig. 2. Vertical distributions of dissolve oxygen concentrations.

Vertical profiles of water temperature, salinity and DO were measured at weekly (stn. 4-12) and at monthly (stn. l-12) intervals in 1992 using an STD and DO meter. Short periodic measurements (every 30 min) were also taken in 1991 and 1994: water temperature, salinity and current velocity at depths of 3 m, 9 m and 15 m below sea level at stn. 8 in September and October of 1991, and water temperature and DO at the depth of l m above the seabed at stn. 8 in August and September of 1994.

RESULTS AND DISCUSSION

OXYGEN DEPLETION

Oxygen depletion occurred in the stratified season. Fig. 2 shows the vertical profile of DO concentration in the bay, Oxygen-deficient water masses were formed in the lower layer of the bay. At stn. 7 (near the fish farms), DO concentrations were less than 1 ppm in the bottom layer. The influences of aquaculture activities on oxygen depletion were estimated by comparing fluctuations of DO concentration at stn. 7 with those at sm. 12 (about 1 km from the fish farms) from April to October of 1992 (Fig. 3). The two stations have almost the same water depth and are equal distance from the mouth of the bay. Although there were no differences in the fluctuations of density (water temperature and salinity) between the two stations, DO concentrations at stn. 7 were lower than those at stn. 12. At stn. 7, oxygen-deficient water masses (less than 3 ppm) appeared in the stratified season (from May to October) and the concentration was occasionally less than 1 ppm at the bottom. On the other hand, at stn. 12, oxygen-deficient water masses only appeared intermittently from June to August, and DO concentration was never less than 1 ppm. These results suggest that aquaculture activity, that is, loading of organic material from the aquaculture area, caused the oxygen depletion.

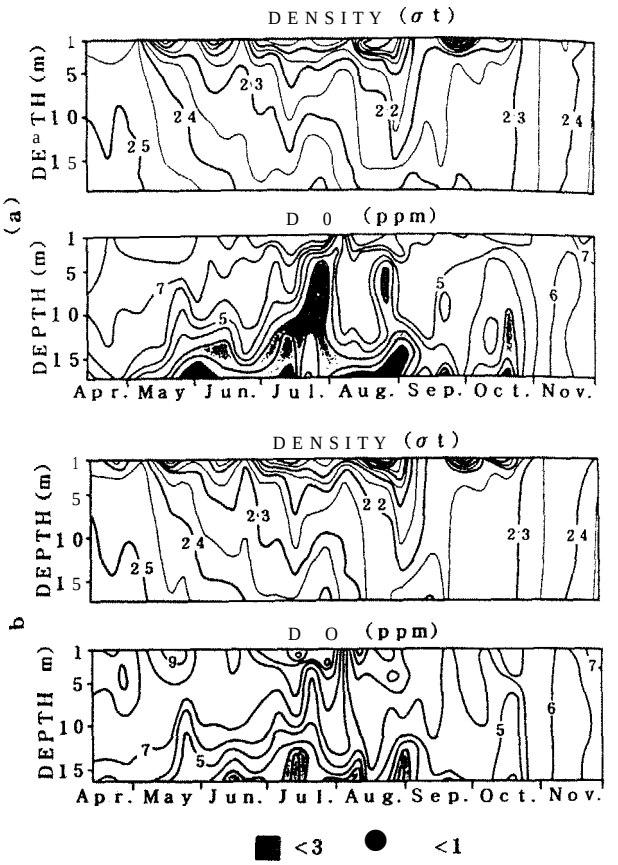

Fig. 3. Seasonal variations of the vertical distributions of density and issolved oxygen at stn. 7 (a) and sm. 12 (b). Sm. 7 is *located near fish farms whereas sm. 12 is located far from fish farms.*

BUDGET OF DO

The oxygen budget in the lower layer of the bay was estimated by using the two-layered box model analysis with the results of field observations. For this model, we defined the inlet as inside the box (stn. 6-l 1), defined the adjacent sea area (sm. 4-5), and separated the box into upper and lower layers at a depth of 8.5 m below sea level. We defined vertical diffusion (D_{12}) and advection (Q_{12}, Q_{12}, and Q_{42}), and disregarded other diffusion and advection (Fig.4).

Vertical diffusion and advection were estimated by considering the conservation of water mass and salinity. The budgets of water mass and salinity are expressed as follows.

$$R+Q_{21}-Q_{13}=0$$

$$Q_{42}-Q_{21}=0$$

$$V_1 dC_1/dt=(C_2-C_1)D_{12}+C_2Q_{21}-C_1Q_{13}$$

$$V_2 dC_2/dt=(C_1-C_2)D_{12}-C_2Q_{21}+C_4Q_{42}$$

Where C_n is the salinity in box-n (n=1, 2, 3, 4), V_n is the volume of box-n, R is the freshwater inflow into box-1, Q_{nm} is the flux of water from box-n to box-m (advection) and D_{12} is the vertical diffusion. If the salinity (C_n) is observed at intervals of dt, the advection and diffusion can be estimated. Using these diffusion and advection estimates. the oxygen budget can be calculated. The oxygen budget in the lower layer (box-2) is as foilows.

$$V_2 dC_2/dt=(C_1-C_2)D_{12}-C_2Q_{21}+C_4Q_{42}-P$$

Here, C_n is the DO concentration in box-n and P is the consumption of DO. This consumption of DO consists of the consumption by water and bottom sediment and respirations of fish. Production of DO by photosynthesis is negligible in the lower layer of the bay.

Fig. 5a shows the vertical diffusion (D_{12}) and the horizontal advection (Q_{42}) estimated by the box model analysis from April to October in 1992. The vertical diffusion was low in July and August and high in September and October. The horizontal advection showed short periodic fluctuations from April to August and got higher in September and October. In the stratified season, the vertical diffusion was low. In autumn, both the vertical diffusion and horizontal advection were high. Fig. 5b shows the consumption rates of DO in the lower layer of the bay; the consumption rate was high in September and October.

The results of the oxygen budget clarified the factors affecting oxygen depletion. The fluctuations of DO concentration at stn. 7 were correlated with vertical diffusion, horizontal advection and consumption rates of DO. In summer, oxygen depletion was caused by low vertical diffusion due to the stratification. In autumn, a very high consumption rate of DO caused oxygen depletion, despite high vertical and horizontal fluxes of oxygen.

The horizontal advection and consumption rate of DO also showed short periodic fluctuations distinct from the seasonal fluctuation. Low DO concentration layer recovered intermittently relative to the short periodic fluctuations of horizontal advection and consumption rates of DO. These short periodic fluctuations were related to the intermittent events of water movements, discussed in the following sections.

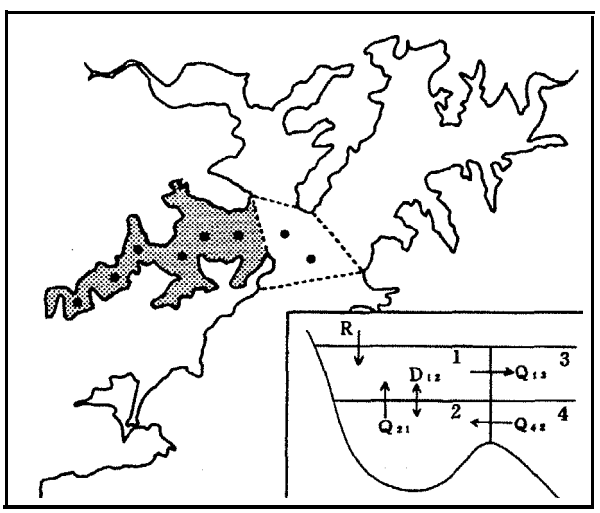

Fig. 4. Schematic view of two-layered box model analysis.

WATER MOVEMENT

Distinctive water movements were found in Gokasho Bay, that is, cold and high-saline water masses intruded into the bay intermittently. Fig. 6 shows the time series of current velocity, water temperature and salinity at stn. 8. Arrows in the figure denote the times when cold and high-saline water masses outside the bay intruded into the lower layer of the bay.

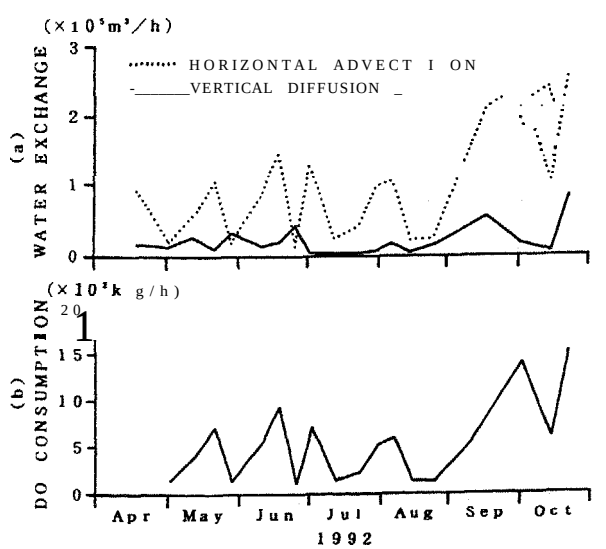

Fig. 5. (a) Seasonal variations of horizontal advection and vertical diffusion as estimated by the two-layered box model analysis. (b) Seasonal variations in the consumption rate of dissolved oxygen.

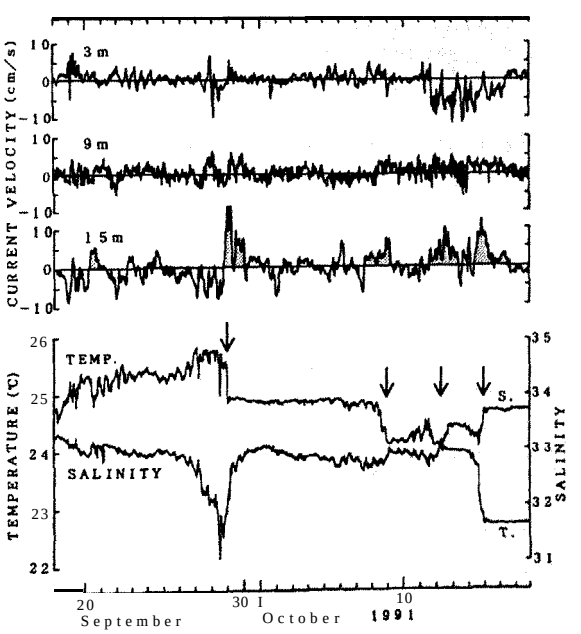

Fig. 6. Time series of current velocity at depths of 3 m, 9 m and 15 m at stn. 8. Water temperature and salinity at depth of 15 m below sea level at stn. 8. Positive values of current velocity denote the inflow. Arrows denote intrusion events.

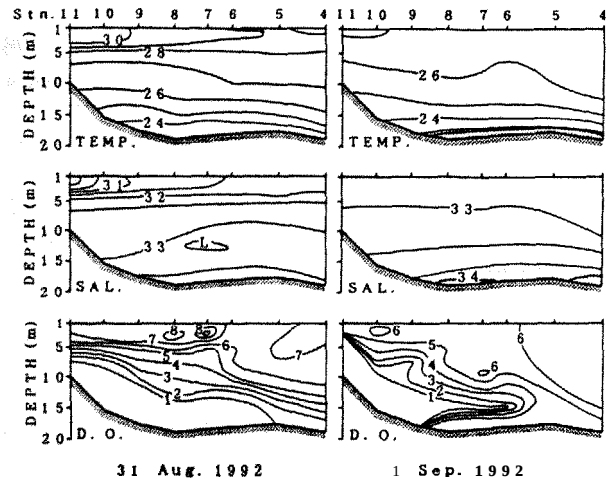

Fig. 7. Vertical *distributions of water temperature, salinity and dissolved oxygen. Stippled areas &note the cold and high-saline water masses that intruded into the bay.*

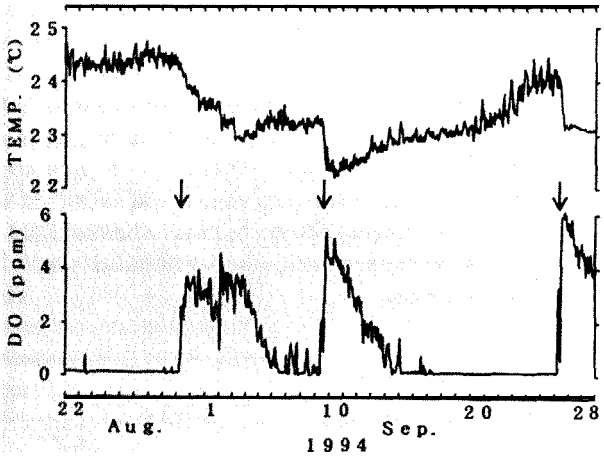

Fig. 8. Time series of water tempemture and dissolved oxygen concentration in the lower layer at stn. 8.

These intrusions occur as a consequence of the coastal upwelling induced by alongshore wind (Abo et *al.* 1996). In alongshore (westerly) winds, the Coriolis force causes the surface water outside the bay to move away from the shore (Ekman transport). The surface water moving away from the shore is replaced by deeper water that upwells close to shore, and the thermocline outside the bay ascends (coastal upwelling). With intermittent winds, the thermocline outside the bay fluctuates vertically, responding to the wind. When the ascent of the thermocline outside the bay reaches a shallower layer than the depth of the mouth of the bay, water masses below the thermocline are able to intrude into the bay.

EFFECTS OF WATER MOVEMENT ON DO

DO concentrations showed short periodic fluctuations. The low oxygen water masses in the lower layer disappeared intermittently (Fig. 3a). These intermittent renewals of low oxygen water masses were caused by the short periodic water movements, that is, by the intermittent intrusions of coastal water masses from outside the bay.

Since DO concentrations outside the bay were higher than those in the lower layer of the bay in the stratified season, the intrusions added a supply of DO into the lower layer of the bay. Fig. 7 shows the supply of DO due to the intrusions of cold and high-saline water masses outside the bay. On August 31, water temperature and salinity in the bay were higher than 23.4flC and lower than 33.8 psu (practical salinity unit), respectively. There was a low oxy gen water mass (less than 1 ppm) in the lower layer of the bay. On September 7, a water mass having low temperature, high salinity, and high DO concentration (more than 4 ppm) intruded into the lower layer of the bay. DO was renewed due to this intrusion.

Such intrusions and subsequent renewals of DO occurred intermittently. Fig. 8 shows the sudden recoveries of DO in the lower layer of the bay (stn. 8) due to the intrusions of cold water masses from outside the bay. As a result, oxygen-deficient water masses disappeared temporarily after the intrusions. It is concluded that such intrusions were significant factors in the renewal of oxygen in the lower layer of Gokasho Bay.

SUMMARY

Oxygen-deficient water masses were formed in the lower layer of Gokasho Bay in the stratified season. The results of the DO budget suggest that summer oxygen depletion is caused by low vertical mixing, due to a strong stratification; whereas, in autumn, a very high consumption rate of DO caused oxygen depletion despite a higher horizontal advection.

Water masses below the pycnocline outside the bay, which have high density and high DO concentration, in-

truded into the bay. The intrusions induced a flux of DO into the lower layer of the bay; low oxygen concentrations disappeared temporarily after the intrusion. The intrusions were significant factors in supplying oxygen to the lower layer of the bay. Thus, the effects of water movements must be considered when studying oxygen depletion in coastal areas such as Gokasho Bay.

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Concepts of Herd Health for Shrimp

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ABSTRACT

Identifying a risk factor as a cause requires the demonstration of an association, correct time order and coherent findings, relative to what is known about the factor and outcome. We demonstrate how these requirements impact epidemiologic investigations into shrimp mortality. Quantifying mortality at intervals throughout growout has been the major problem with on-farm studies. In studies using mortality estimates at the end of production cycles, the correlation of outcome within ponds and within farms reduced our ability to extrapolate results. Measurement error for risk factors has been a secondary problem. Mortality can be measured accurately if the study's internal time component is a complete production cycle. Because risk factors frequently do not change during a production cycle, the association between risk factors and mortality can be evaluated, assuming a random effect of production cycle, using random-effects logistic regression. It is likely that the farm will modify the cause and effect relationship and future studies should sample multiple farms to increase external validity.

INTRODUCTION

Epidemiologists study the cause of disease in its natural setting. It is a logical extension that a veterinarian studying herd health for shrimp farming must study shrimp diseases using actual farm observations. Epidemiologists avoid defining the word cause, preferring to use words such as determinants, exposures and risk factors. Alternatively, they categorize causes as direct or indirect; necessary or sufficient; and single or multiple, rather than defining cause. In an effort to be pragmatic, Susser (1991) has defined cause as "something that makes a difference" or alters an outcome.

The philosophical view of what constitutes a cause has not been so pragmatic (Rothman 1986). However, some consensus has emerged from the arguments of philosophers. Epidemiologists concur that Koch's postulates are much too rigid (Susser 1991). Epidemiologists also agree that causes must be hypothesized and then tested for rejection but are never accepted as proven (Susser 1991), thus an epidemiologist will be well served by an iconoclastic point of view. Epidemiologic methods are therefore not very friendly. A brave soul will hypothesize a cause for shrimp dying, and epidemiologists try to disprove the hypothesis. After an hypothesized causal relationship survives the challenge of repeated testing, as the cigarette smoking/mortality relationship did (Eysenck 1991), the risk factor becomes accepted as a cause of mortality. Further sophistication, however, will challenge the hypothesis as our understanding grows and we get closer to understanding the deterministic model. For example, cigarette smoking is no longer considered a cause of mortality by epidemiologists because more specific carcinogens found in cigarette smoke have been identified. Epidemiologists use three rules to reject an hypothesized cause (Susser 1991). The first is association. If a risk factor does not occur more frequently in diseased animals, then it is rejected as a cause. The second is time order. If the risk factor does not precede the outcome, it is rejected. The third criteria used is common sense or coherence, in all its forms. Risk factors are often rejected based on what is known or believed about the cause and outcome.

In epidemiology, two outcomes are frequently studied: morbidity and mortality. The study of morbidity for shrimp culture can be mediated quite effectively by using growth rate as a surrogate measure of wellness (Thompson et *al.* 1994b). While slow growing shrimp may feel quite well and never miss a day's work, most producers will accept growth rate as a measure of morbidity because of its correlation to their economic health. However, the study of the causes of shrimp mortality has been fraught with difficulties. There is no lack of hypothesized causes but hypothesis testing is difficult. The objective of this review is to present and discuss examples illustrating the difficulties in hypothesis testing for shrimp mortality from on-farm observations. We present and discuss one example for each of the three criteria for causation: association, time order, and coherence of results.

EXAMPLE 1: ASSOCIATION

INTRODUCTION

Necrotizing hepatopancreatitis (NHP) has been an im-

portant disease of shrimp culture in Texas since it was first recognized in 1985 (Frelier *et* al. 1992). The disease has resulted in mass mortalities of *Penaeus vannamei* in commercial growout ponds. The etiologic agent has been shown to be a pleomorphic Rickettsia-like intracellular bacterium (Frelier *et al.* 1992). Previous studies have shown the disease to occur at high salinity (20 to 40 ppt), but the role of salinity remains unclear (Frelier *et al.* 1993). The objective of this study was to describe the association among pond differences in salinity and mortality in 1993 on a Texas farm for which the disease agent was endemic.

MATERIALS AND METHODS

A production unit in south Texas that had suffered recurrent problems of NHP was surveyed for histological lesions of NHP during the 1993 production season. Salinity was recorded in each pond on a weekly basis. The mean was calculated for each pond and classified as high salinity if greater than 35 ppt and low salinity if less than 35 ppt. Mortality was calculated as a proportion, with the numerator the difference between the number of shrimp stocked into the pond minus the number harvested from the pond. The denominator was the number of shrimp stocked into the pond. Analysis of the association between salinity and mortality while accounting for clustering of mortality within the pond was performed using various methods described by Donner (1993).

RESULTS

DISCUSSION

The choice of an appropriate statistical method to test for association was considered. The outcome, mortality, is highly clustered within ponds creating extrabinomial variation. Similar effects are well-described on the smaller scale and are often referred to as litter effects (Donner 1993). The use of the Pearson chi-square statistic ignores the clustering within ponds; this statistic requires that the outcomes be independent and its value overinflates the statistical significance. The t-test methods will evaluate a single measurement of survival from each pond. This will account for clustering but will almost surely violate the assumptions that the variances from each group are normal and homoscedastic. The non-parametric approach is valid but is inefficient because it ignores the number of shrimp in each pond and the magnitude of the differences. Also, the size of the effect is not intuitively useful.

Use of random effects models has intuitive appeal (Curtis *et* al. 1993). The effects of "pond" can he modeled as a random effect, represented by a distribution of pond effects. By so doing, the variance and standard errors of the estimates are inflated to account for the extrabinomial variation. Software Egret® has been marketed to model such random effects. However, the models were not developed with shrimp ponds in mind and most of the fitting algorithms failed with our example. We achieved convergence using the beta-binomial random effects model in Egret®. By repeating the fitting with different initial values, we observed multiple maxima (several possible solutions). It was straightforward to pick the best of the observed solutions by selecting the model with the lowest deviance (Hosmer and Lemeshow 1989). However, the possibility of an alternative, superior solution, exists. We found no adequate single test for association with the described example.

The evidence of an association between salinity and mortality was strong even though the difference between high and low salinity definitions was very small. Evidence for the association is convincing, but how strong is the evidence that salinity is a cause? Time order has not been established. It is possible that mortality precedes high salinity. High mortality may have been a specific condition or associated with a condition that preceded an altered rate of exchange of water. It is unlikely that time order can be established until accurate measures of mortality during a production cycle are possible. The size of the effect makes the likelihood of the association being explained by a bias less likely than if the size of the effect was small.

Proposing salinity as a cause of mortality in the presence of NHP lesions lacks sophistication. What about salinity as a cause of death (Lester and Pante 1992)? Is it its effect on osmoregulation? Is it a stress that reduces immunity? Is it a marker for a change in pond biota with a proliferation of a specific intermediate host? The evidence for coherence in all its forms can be improved with further study. With existing limitations, it is unlikely that we can strengthen the time-order relationship with pond observations. Tank observations may not be applicable to studies on disease in production systems. It is conceivable that increasing salinity in a laboratory tank will not constitute stress but in a pond, high salinity with a combination of other factors will. Tank studies may remove intermediate hosts from the other pond biota. The answer to elucidating the causal mechanism lies in the posing and investigating of more specific and sophisticated epidemiologic hypotheses that will add to our knowledge of the other contributing factors. In the meantime, in a pragmatic world, we must consider salinity as a cause of NHP (Susser 1991).

EXAMPLE 2: TIME ORDER

INTRODUCTION

Vibrio spp. are considered the most important bacterial pathogens in shrimp culture (Vera *et al.* 1992), *and* are important causes of enteritis in humans. Special concern must be placed on pathogens of human concern that may appear in harvested products. Like bacterial pathogens in

Table 1. Summary of statistical testing of the significance and size of an association.

Method	Test statistic	P	Effect size
Pearson hi-square	4.5×10^5	P<0.00001	Odds ratio = **7.0**
Student's t-test (t)	3.95	P=0.00 1	Difference of means = 24%
t-test (transformed) (t)	3.86	P=0.002	Difference of means = 24%
Wilcoxen rank-sum (w)	215	P=0.00 1	Non-parametric
Random effects model (likelihood ratio chi-square)	26.0	P<0.0000 1	Odds ratio = 5.0

Table 2. Odds ratios for high mortality risk over a 1 wk period.

Variable	Odds ratio (95 % confidence interval for odds ratio)	P
Pond		P<0.001
Weight (increase of 1 g)	0.94 (0.90 to 0.98)	P<0.01
Density	0.19 (0.12 to 0.31)	P<0.0001
$(\text{Density})^2$	1.08 (1.05 to 1.11)	P<0.0001
Full moon	0.52 (0.32 to 0.84)	P<0.01
Low dissolved oxygen	1.61 (1.00 to 2.59)	P<0.05
High *Vibrio* concentration in HP	1.32 (0.63 to 2.79)	P=0.5
DO2 x high [*Vibrio*]	0.82 (0.48 to 2.09)	P=0.7

Table 3. Univariate association between low dissolved oxygen concentration and a high mortality risk. The overall crude odds ratio was 1.27.

		Class of dissolved oxygen concentration		
		Low	**High**	**Total**
Risk of mortality	High	167	201	368
	Low	193	294	487
	Total	360	495	855

Table 4. Association between low dissolved oxygen concentration and a high mortality risk after correction for non-differential misclassification of mortality with a sensitivity of 0.6 and specificity of 0.6. The overall odds ratio was 15.1.

		Class of dissolved oxygen concentration		
		Low	**High**	**Total**
Risk of mortality	High	115	15	130
	Low	245	480	725
	Total	360	495	855

other farmed species (Martin et al. 1987), these bacteria are ubiquitous and disease is triggered by stress (Lightner 1993). Stress is frequently implicated by epidemiologists as an initiating factor for bacterial disease in livestock. ***Stress*** is often poorly understood in its pathogenesis but epidemiologists frequently compare surrogate measures of stress to study its role as an epidemiologic factor, Dissolved oxygen is the most frequently measured variable

in production ponds (Thompson et *al.* 1994a) and it may serve as a suitable surrogate measure of stress. Concentrations of *Vibrio* spp. vary widely among ponds, and also temporally within ponds. Because of the variance that occurs within pond-cycles, analysis of the *Vibrio*/dissolved oxygen interaction will require a selection of an internal time component and periodic measurement of mortality during a production cycle. The objective of this study was to determine if the presence of *Vibrio* spp. in the hepatopancreas was associated with mortality and if this association was modified by low dissolved oxygen concentrations.

MATERIALS AND METHODS

Dissolved oxygen concentrations were performed as close to 6 am. as possible each day. The mean was calculated for the seven measurements of the wk. For categorical analyses, low dissolved oxygen concentration was defined as less than 5.2 mg/L (the median) and high concentration as a mean greater than or equal to 5.2 mg/L. To quantify the number of *Vibrio* colonies, 9 ml of 0.9% saline were mixed with 1 g of hepatopancreas. The mixture was macerated, mixed and a 0.1 ml aliquot was plated on TCBS agar. At 24 h, the number of colonies was counted and classified as high if greater than the median observation (500 colonies) and low if equal to or less than 500 colonies. To &fine the pond's population, a cast net that sampled approximately 1 m^2 was cast at 54 points. The average number caught was multiplied by 10,000 to give the number per ha and then multiplied by the number of ha. Pond variables and pond population estimates were stored in a Lotus data base and retrieved at the end of the study. To calculate the risk function, the force of mortality was calculated (Kleinbaum *et al.* 1982). For logistic analyses, the force of mortality was classified as high if positive and low if zero or negative. Multiple logistic regression was performed regressing the outcome high vs. low mortality against pond; pond density and the square of the pond density: phase of the moon; dissolved oxygen; hepatopancreas *Vibrio* concentration and the dissolved oxygen and *Vibrio* interaction. The phase of the moon was modeled as a risk factor if the full moon occurred during the observation wk. Dissolved oxygen and number of *Vibrio* colonies were modeled using the classification variables.

RESULTS

Weeks with high mortality were associated with pond, weight, density and the density squared, and occurrence of a full moon during the wk (Table 2). High mortality was also related to the classification of oxygen concentration but not to the number of *Vibrio* colony forming units (CFUs) nor the interaction between dissolved oxygen and *Vibrio* CFUs. A univariate table for the risk of low dissolved oxygen was created (Table 3), which demonstrated that the crude odds ratio for low dissolved oxygen was 1.27. This table was corrected for a non-differential misclassification bias of sensitivity of 0.6 and specificity of 0.6. The true, population crude odds ration can be shown to be 15.1 (Table 4).

DISCUSSION

We have failed to demonstrate a significant effect modification between *Mbrio* and dissolved oxygen concentration. This failure occurred with methods designed to estimate a population size at the beginning and ending of each week. These measurements were made with error that produced a large misclassification bias (Brenner and Blettner 1993). We are not certain of the degree of misclassification but any rise in population estimates constitutes a misclassification and counting these demonstrates the sensitivity of classification to be approximately equal to 0.6. We also believe that if we were classifying a realistic, low mortality risk (0.05/wk), the classification would also have a very low specificity. If the measurement error is centered on zero and normally distributed, we would expect the same percent of errors for both high and low mortality estimates and the specificity would also be equal to approximately 0.6. We show that using a sensitivity of 0.6 and specificity of 0.6 for the misclassification would have a very large bias of the odds ratios toward the null value, We argue that this misclassification biases all potential predictors of mortality toward the null value.

This study was doomed to failure for the detection of relatively small odds ratio. It can be concluded that the odds ratios determined for shrimp density, lunar phase and low dissolved oxygen concentrations were either extremely large or the product of other biases. Our calculations for corrected odds ratios were based on the assumption that the misclassification of mortality was not related to any of the predictors. This assumption could not be validated (Flegal et *al.* 1991). It is quite possible that estimates may also be related to shrimp density and weight because of shrimp behavior in lunar phases (Griffith and Wigglesworth 1993). It is also possible that low dissolved oxygen concentrations reduce the likelihood of capturing shrimp. In general, the uncertainty of the size of the odds ratios and the size of the misclassification and whether the classification is non-differential or not makes the study design unworkable (Flegal *et* al. 1991). We believe that measurement error of population estimates during a production cycle makes the study of small or moderate risks untenable.

EXAMPLE 3: COHERENCE

INTRODUCTION

Taura syndrome was first recognized as a cause of mortality in June 1992 on shrimp farms near the mouth of the Taura River in Ecuador (Brock et *al.* 1994). Since then,

the disease has been diagnosed elsewhere in Ecuador, Peru, Columbia, Honduras and Hawaii (Brock et al. 1994). In May and June of 1995, five Texas shrimp farms suffered devastating losses in association with a histopathologic diagnosis of Taura syndrome virus (TSV). Because the virus had been considered exotic to Texas, an epidemiologic investigation was conducted to evaluate the most likely of a number of specific possible sources of the virus using case-control methodology (Fonseca and Armenian 199 1). Of special concern was the possibility that the spread of TSV was mediated through transport of infected post-larvae.

MATERIALS AND METHODS

All major shrimp growout production units in Texas (n=6) and an experimental unit were visited and evaluated for exposure to the potential sources of infection. Identification of unexpected mortality was based on a questionnaire delivered by the lead investigator to the farm owners or managers. Each unit was evaluated for the presence of TSV based on histopathology. Cases were farms with unexpected mortality and a histopathologic diagnosis of TSV. Exposure to the potential sources was based on historical data and inspection by the lead investigator. Associations between potential infection sources (factors) and case status were evaluated with Fisher's Exact Test.

RESULTS

Taura syndrome was diagnosed in five of six commercial growout farms and was absent in the experimental unit. Feed used consisted of six different formulations from multiple batches from two separate feed companies. Water inlets were closed for the majority of the case ponds. It became apparent during the outbreak that new cases occurred irrespective of water flowing into the ponds. The index case farm also supplied larvae to other farms in Texas (and elsewhere). Three case farms received larvae when TSV was active on the index farm (after May 6). This source of larvae did not explain disease at the index farm and at one other case-positive farm. Exposure to airborne factors, wild in the Laguna Madre, was found at all farms but not the experimental unit.

DISCUSSION

Identification of a causal factor requires the demonstration of association, temporal sequence and coherence. However, we must consider that our general grouping of causes into airborne, waterborne, feedborne and animalborne is likely an exhaustive list and to meet our objective we should identify the most likely source. If we hypothetically expand our sample to include 10 more US shrimp farms, all newly sampled farms would be factor negative and case negative. If this sampling did not constitute a bias, we would conclude that the association of transported Post-larvae and TSV were associated; however, the same can be said to be true of exposure to the Laguna Madre and TSV. For temporal sequence, we can say both factors preceded the mortality effect.

In our examination of coherence, we note inconsistencies in the lag (or incubation) periods. Incubation periods have been long recognized asuseful indications of a cause (Sartwell1995). For the three farms receiving larvae as potentially infected, one had a 1 wk incubation period and two had 5 wk incubation periods. The incubation periods for the airborne source can, in contrast, be explained by a south to north migration of the vehicle. Analogy, a form of coherence, also favors the airborne source hypothesis. Within case farms, spread was rapid among ponds with a very short incubation and a short period of mortality. The findings are compatible with a common source airborne spread. This could be explained by multiplication of a virus within an insect host but not by a mechanical vector. If the virus was spread by infected larvae, the hypothesis of airborne-spread must still be true within farms. Because of the rapidity of spread, we propose that the infection source is part of the preferential diet of shrimp such as an insect with both airborne and waterborne life stages. Through analogy and the knowledge that within-farm-spread was airborne, it was shown that airborne-spread was definitely involved. Was the spread by infected post-larvae also involved? The preponderance of other evidence (coherence) says no.

CONCLUSIONS

The studies of shrimp diseases with on-farm observations have been frustrating. We propose the following recommendations for future study.

Concentrate on "fixed" factors as risk factors. Risk factors that are constant throughout a growout cycle by definition must precede mortality so time order cannot be faulty. These factors include: salinity and other environmental variables on most farms; pond size, depth and other physical properties, including aerators; and endemic diseases.

Use random effects models to demonstrate associations. These models can account for extrabinomial variation. Although developed for clusters much smaller than shrimp ponds, these models can be used provided routine diagnostics for regression models are applied.

Increase the number of farms in the sample. Fixed risk factors will usually be constant for all ponds within a farm. Random effects models are used when the outcomes are clustered. A farm becomes a single cluster with a single outcome rate and a single level of fixed risk factor. The need for multiple farms is obvious.

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Diarrhetic Shellfish Toxins Determined by High-Performance Liquid Chromatography-Fluorometry in Mussels, *Mytilus coruscus*, from the Niigata Coast of Japan

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ABSTRACT

Determination of okadaic acid (OA) and its analogues, dinophysistoxin-1 (DTX-1) and dinophysistoxin-3 (DTX-3), in mussels collected from the Niigata coast of Japan was carried out by high-performance liquid chromatography (HPLC) with fluorometric detection after derivatization with 9-anthryldiazomethane. Analyses of toxic material indicated the presence of OA and DTX- 1; however, the toxicity determined by HPLC-fluorometty was far below that estimated from mouse bioassays, suggesting the presence of other toxins.

INTRODUCTION

Diarrhetic shellfish poisoning (DSP) has been recognized as a worldwide problem since it was reported by Yasumoto *et al.* (1978). Among the diarrhetic shellfish (DS) toxins derived from dinoflagellates, *Dinophysis* spp. and *Prorocentrum* spp. (Lee et *al.* 1989). the most important toxins responsible for diarrheal symptoms are okadaic acid (OA) and its derivatives, dinophysistoxin- 1 (DTX- 1) and dinophysistoxin-3 @TX-3) (Lee *et al.* 1987). In 1994, a DSP outbreak occurred along the Niigata coastal area, Japan. Shellfish harvesting was prohibited from May 16 to August 2. The present paper reports results of DS-toxin analysis by high-performance liquid chromatography (HPLC)-fluorometry to elucidate the nature of the toxic DSP compounds in mussels collected from the Niigata coastal area. Determination by HPLC-fluorometry of the free fatty acids (FFAs) which have been suggested to interfere with mouse bioassays (Takagi et al. 1984) is also described.

MATERIALS AND METHODS

Mussels, *Mytilus coruscus*, were collected from the Niigata coastal area during the period May 30 to June 27, 1994. Five individual mussels were used for each analysis. Acetone extraction of DSP toxins from the midgut glands of mussels and the following mouse bioassays were carried out using the procedure described by Yasumoto (198 1). Analyses of OA and DTX- 1 in the acetone extracts by HPLC-fluorometry were carried out according to the method of Lee *et* al. (1987). Determination of DTX3 was carried out by the detection of DTX-1 obtained from DTX-3 via alkaline hydrolysis (Suzuki 1994) after separation by partitioning between 80% methanol (OA and DTX-1) and n-hexane (DTX-3). Fluorescent peaks corresponding to the 9-anthryldiazomethane (ADAM) derivatives of OA and DTX- 1 were confirmed by a second HPLC-fluorometry using a Capcell Pak CN SG120 column (4.6 mm ID x 250 mm; Shiseido, Tokyo, Japan) as described by Zhao et *al.* (1993). Further confirmation of the OA and DTX-1 peaks was carried out by HPLC-fluorometry using a LiChrosorb RP- 18 column (4.0 mm ID x 250 mm; Merck, Darmstadt, Germany), with acetonitxile-methanol-water (8: 1: 1, v/v/v) as the mobile phase and a flow rate of 1.1 ml/min at 35°C. Determination of the peak area corresponding to OA and DTX- 1 was carried out on the second HPLC run equipped with a Capcell Pak CN SG120 column. Determination of FFAs by HPLC-fluorometry was carried out according to the method of Suzuki (1994). The toxicity of the FFAs was calculated from data reported by Takagi et al. (1984). One liter seawater samples were collected at 8 m depth at the monitoring station and fixed with formalin (5%). The solution was concentrated to 10 ml for counting and identification of *Dinophysis* spp. by microscopy.

RESULTS AND DISCUSSION

Table 1 shows the mouse bioassay results for the midgut glands of mussels during the DSP outbreak and cell densities (cells/L) of *Dinophysis spp.* from 8 m depth where the mussels were harvested The DSP outbreak on May 9,

Table 1. Results of mouse bioassays and cell densities (cells/L) of *Dinophysis* species collected from the Niigata coastal area in 1994

Date		Bioassay Results[1] (MU [2]/g)	Species *D. fortii*	*D. mitra*	*D. rudgei*	Other spp.[3]
May	9	0.5 - 1.0	170	—	—	—
May	16	0.5 - 1.0	10	—	10	—
May	22	0.3 - 0.5	10	—	—	—
May	30	0.5 - 1.0	—	—	—	—
June	6	0.5 - 1.0	10	—	—	—
June	14	0.5 - 1.0	—	—	—	—
June	20	0.5 - 1.0	—	—	—	—
June	27	0.5 - 1.0	—	—	—	—
July	8	<0.3	—	30	—	—
July	19	<0.3	—	—	—	—
July	26	<0.3	—	—	—	—

[1]Toxicity of the mussel midgut glands.
[2]Mouse unit (MU); one MU is defined as the amount of toxins to kill a male mouse of ddY strain of 20 g body weight in 24 h.
[3]*D. acuminata, D. norvegica, D. infundibulus, D. rotundata, D. tripos* and *D. caudata.*

1994, occurred with the appearance of *D. fortii*, suggesting that *D. fortii* was responsible for the observed toxicity of mussels along the Niigata coast in 1994. The toxicity of mussels remained above the quarantine level of 0.5 mouse unit (MU)/g of midgut glands from May 9 to June 27.

Fig. 1 shows a representative HPLC profile of the ADAM derivatives of extracts obtained from the midgut glands of mussels. Peaks corresponding to OA and DTX-1 were detected, whereas the fluorescence intensity of respective peaks was very small (Fig. 1). Peaks corresponding to OA and DTX-1 were collected from the outlet of the fluoromonitor, and each concentrated fraction was verified by the two different second chromatography runs, using a Capcell Pak CN SG120 (Zhao *et al.* 1993) or LiChrosorb RP-18 column. Chromatograms of the fractions of OA and DTX-1 collected from the first HPLC indicated the presence of peaks with retention times exactly matching those obtained from the ADAM derivatives of the standard toxins (Figs. 2 , 3), indicating that the mussels under investigation were contaminated by OA and DTX-1. In samples of toxic Japanese mussels, the most prominent DS toxin is usually DTX-1 (Kumagai *et al.* 1986). The presence of OA in Japanese mussels was confirmed by HPLC-fluorometry. DTX-1 hydrolyzed from DTX-3 was not detected in the present HPLC-fluorometry, indicating that mussels analyzed did not contain DTX-3.

Table 2 gives the concentrations and toxicities of OA and DTX-1 in the midgut glands of mussels as determined by HPLC-fluorometry. The sum of the toxicity of OA and DTX-1 was 0.02-0.13 MU/g of midgut glands. The tox-

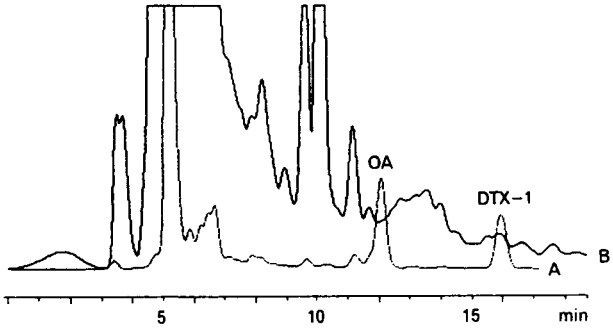

Fig. 1. HPLC profiles of the ADAM derivatives on the Develosil ODS-5 column. (A) purified OA and DTX-1; (B) toxic mussel midgut gland (Niigata, June 1994). Conditions: mobile phase, acetonitrile-methanol-water (8:1:1, v/v/v); monitor, excitation 365 nm, emission 412 nm; flow rate, 1.1 ml/min; temperature, 35°C.

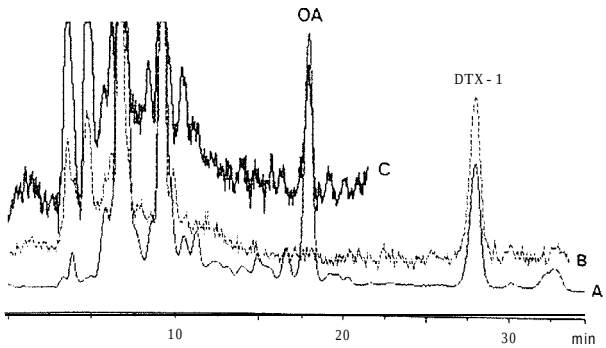

Fig. 2. HPLC profiles of the ADAM derivatives on the Capcell Pak CN SG120 column. (A) purijied OA and DTX-I; (B) fraction corresponding to DTX-1 in HPLC on the Develosil ODS-5 column; (C) fraction corresponding to OA in HPLC on the Develosil ODS-5 column. Conditions: mobile phase, acetonitrile-water (53:47, v/v); monitor; excitation 365 nm, emission 412 nm; flow rate, 1.1 ml/min; temperature, 35°C.

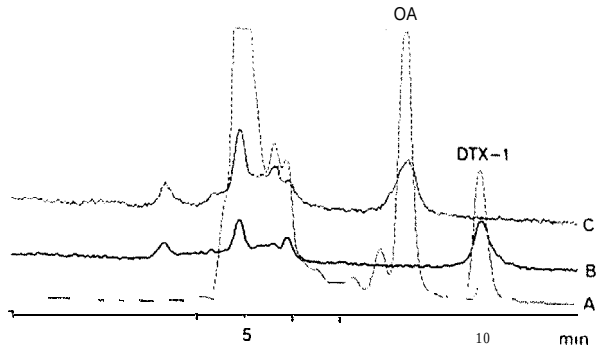

Fig. 3. HPLC profiles of the ADAM derivatives on the LiChrosorb RP-18 column. (A) purified OA and DTX-I; (B) fraction corresponding to DTX-1 in HPLC on the Develosil ODS-5 column; (C) fraction corresponding to OA in HPLC on the Develosil ODS-5 column. Conditions: mobile phase, acetonitrile-methanol-water (8:1:1, v/v); monitor, excitation 365 nm, emission 412 nm; flow rate, I.1 ml/min; temperature, 35°C.

icities determined by HPLC-fluorometry were clearly lower than those obtained by mouse bioassays. The slight decrease in toxin content in the midgut glands of mussels after June 14 correlates with the disappearance of *D. fortii* after that date (Table 1).

The FFA content is also shown in Table 2. Although a marked increase in FFA content was observed on June 20 and 27, the FFA content was not sufficient to give positive results (>0.5 MU/g) in the mouse bioassays.

The presence of OA and DTX-1 in toxic mussels collected from the Niigata coast was confirmed by HPLC-fluorometry, but the sum of the toxicities determined by HPLC-fluorometry was too low to account for the mouse lethality. FFA contents were also insufficient to interfere with the mouse bioassay. These results indicate the presence of other DSP toxin(s), including pectenotoxins and yessotoxins (Yasumoto and Murata 1990), and suggest the need to analyze for other DSP toxins during routine instrument methods of monitoring for the toxicity of mussels in this area.

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Table 2. Diarrhetic shellfish toxin and free fatty acid contants in the midgut glands of mussels collected from the Niigata coastal area in 1994

Date		Bioassay results (MU/g)	OA (μg/g)	OA (MU/g[1])	DTX-1 (μg/g)	DTX-1 (MU/g[1])	FFA (μg/g)	FFA (MU/g[1])
May	9	0.5 - 1.0	ND[2]	ND	0.16	0.05	666	0.02
June	6	0.5 - 1.0	ND	ND	0.16	0.05	443	0.02
June	14	0.5 - 1.0	0.05	0.01	0.37	0.12	523	0.02
June	20	0.5 - 1.0	0.01	<0.01	0.11	0.03	2197	0.11
June	27	0.5 - 1.0	ND	ND	0.07	0.02	6322	0.37

DTX-3 not detected in any sample.

[1]Calculated on the basis of specific activities of 4.0 and 3.2 μg/mouse unit (MU) for OA and DTX-1, respectively.

[2]Not detected.

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Analytical Methods for Diarrhetic Shellfish Poisoning (DSP) Toxins and a Study of Toxin Production by *Prorocentrum lima in* Culture

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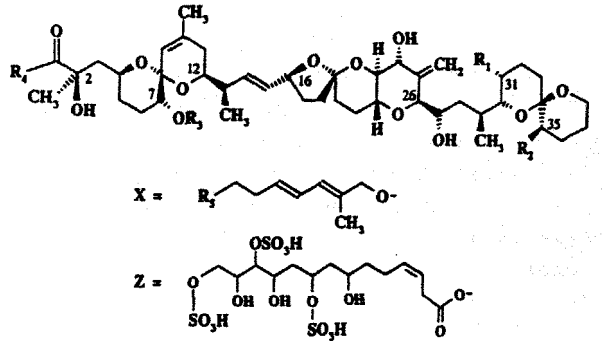
ABSTRACT

Incidents of diarrhetic shellfish poisoning (DSP) pose a serious threat to the aquaculture industry. DSP results from consumption of shellfish contaminated with toxic dinoflagellates such as species of *Dinophysis* and *Prorocentrum*. During a study to determine the kinetics of production and excretion of DSP toxins in culture by one such organism, *Prorocentrum lima*, a number of new ester derivatives of okadaic acid and DTX1 were discovered. It was also found that enzymatically-catalyzed hydrolysis and methanolysis reactions were occurring during simple extractions with aqueous methanol. Extraction and analysis methods developed to determine toxin concentrations in cells and in the medium will be valuable for future studies on the uptake and depuration of DSP toxins by shellfish, and for protection of public health.

INTRODUCTION

Diarrhetic shellfish poisoning (DSP) is a severe gastrointestinal illness resulting from consumption of shellfish contaminated with toxigenic dinoflagellates, such as certain *Dinophysis* and *Prorocentrum* species (Yasumoto and Murata 1990). The main toxins responsible for DSP are okadaic acid (OA) and the dinophysistoxins, DTX1 and DTX2 (l-3, see Fig. 1). These compounds have been shown to be potent phosphatase inhibitors, a property which can cause inflammation of the intestinal tract and diarrhea. In addition, OA and DTX1 have been shown to have tumor-promoting activity. A number of naturally occurring derivatives of these toxins has also been identified. The 7-O-acylated (C_{14} to C_{18}) derivatives (4) of l-3 have been found in shellfish tissue and designated as DTX3 (Yasumoto *et al. 1989,* Marr *et al.* 1992b). However, DTX3 toxins have not been detected in microalgae, suggesting that they are products of shellfish metabolism (Yasumoto *et al.* 1989). Several ester derivatives of OA, such as diol-ester 5, have been isolated from *P. lima* and *P. maculosum* (Yasumoto *et al.* 1989, Hu *et al.* 1992). Although the ester derivatives do not appear to be phosphatase inhibitors, they have the potential to be hydrolyzed readily in the digestive tract to yield an active parent DSP toxin. Recently, a water-soluble DSP toxin (DTX4) was isolated from an eastern Canadian strain of *P. lima* (Hu *et al.* 1995). This compound is a complicated derivative of OA, in which the primary hydroxyl of diol-ester 5 is esterified with a trisulfated end group.

In this study we report on the DSP toxins produced by *the* dinoflagellate *P. lima in* culture. A number of new ester derivatives of OA and DTX1 were identified, and it was found that enzymatically-catalyzed transformations were occurring during simple extractions with aqueous methanol. New analytical procedures are demonstrated for the accurate determination of toxin concentrations in cells and in the medium.



	R_1	R_2	R_3	R_4	R_5	
1	CH_3	H	H	OH	—	**OA**
2	CH_3	CH_3	H	OH	—	**DTX1**
3	H	CH_3	H	OH	—	**DTX2**
4	(H or CH_3)		Acyl	OH	—	**DTX3**
5	CH_3	H	H	X	OH	**OA diol-ester**
6	CH3.	H	H	X	Z	**DTX4**

Fig. 1. Structures of okadaic acid (Oh) and same of its known natural& occurring derivatives.

MATERIALS AND METHODS

CULTURING PROCEDURES

The strain of *P.* lima used in this study, its isolation, culture medium and growth conditions were reported previously (Marr et al. 1992a, Jackson et al. 1993, McLachlan *et al. 1995).*

SAMPLE PREPARATION

Aliquots (50 ml) of *P. lima* culture were transferred to 50-ml plastic centrifuge tubes and centrifuged for 10 min at 6600 x g. The supernatants were decanted without disturbing the cell pellets. Several different extraction methods were investigated, the principal ones being:

Method 1 (80% methanol): Each cell pellet was resuspended in 2 ml of methanol/water (8:2) and sonicated for 1 min in pulse mode (50% duty cycle, 375 W) while cooling in an ice bath. After centrifugation for 10 min at 6600 x g, the supematant was decanted. The pellet was rinsed twice (vortex mixing, centrifugation) with 1 ml methanol/water (8:2). Supernatants were combined and adjusted to 5.0 ml. Extracts were passed through a 0.45-μm filter prior to analysis.

Method 2 (French press): Four cell pellets were each resuspended in 0.2 ml 50 mM TrisHCl pH 7.4, combined and passed through a chilled French press at pressures >10 Kpsi. A 1 ml aliquot of buffer was used to wash remaining residues through the press and the sample was then brought to 2.0 ml with buffer. Aliquots (0.5 ml) were mixed with 2.0 ml methanol and processed as in Method 1.

Method 3 (freeze/thaw): Each cell pellet was resuspended in 0.5 ml of TrisHCl buffer and immersed in liquid nitrogen. The sample was allowed to thaw at room temperature and left in the dark for 24 h. Then 2 ml of methanol were added and the sample was sonicated and extracted as in Method 1.

Method 4 (boiling): Each cell pellet was resuspended in 0.5 ml of TrisHCl buffer and immersed in boiling water for 3 min. Following this, 2 ml of methanol were added followed by sonication and extraction as in Method 1.

CHEMICAL ANALYSES

Analyses of 1-3 and 5 were performed by positive ion-spray liquid chromatography-mass spectrometry (LC-MS) and of 1-3 by LC with fluorescence detection (FLD) after derivatization with anthryldiazomethane (ADAM), as previously reported (Quilliam 1995). The DTX4 and related toxins were analyzed using the negative ion mode, a 2 x 150 mm column packed *with 5* mm Zorbax Rx-C8, 0.2 ml/min flow rate and gradient elution with an aqueous acetonitrile-ammonium acetate (1 mM, pH 7) mobile phase programmed from 20% to 50% acetonitrile over 15 min. Compounds 5 and 6 were also analyzed by LC with UV detection at 238 nm using the same column and mobile phase.

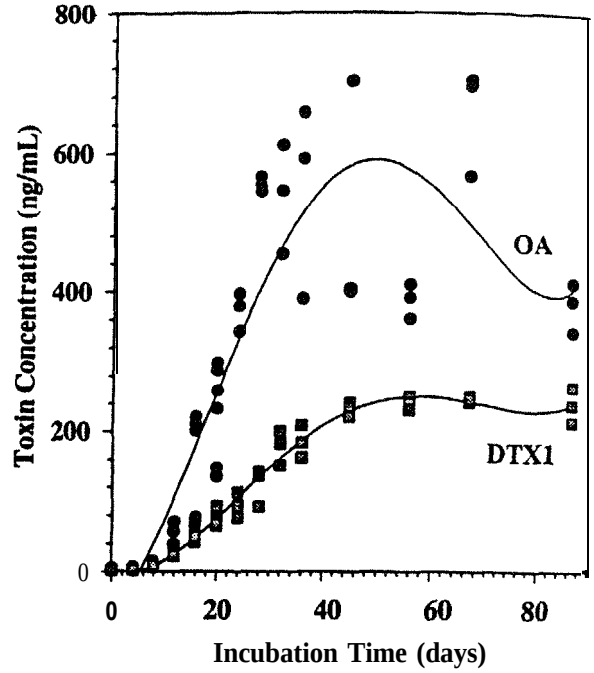


Fig. 2. Intracellular toxin production in P. lima *measured by LC-FLD analyses. Samples were extracted in 80% methanol and then derivatized* with ADAM.

RESULTS AND DISCUSSION

Published extraction methods for DSP toxins in plankton samples have used solvents such as methanol, acetone and chloroform. Initially, we sonicated cells isolated by centrifugation in aqueous 80% methanol and followed this by LC-FLD analysis of the ADAM derivatives. This gave excellent reproducibility with replicate subsamples of a single culture in early growth phase. However, erratic results in the cellular concentration of OA (but not DTX1) were observed as the methods were applied in a study of the rate of toxin production in batch culture. Results of one such experiment are shown in Fig. 2. It was evident from literature reports that others had also experienced erratic quantitative results in such experiments (Jackson *et* al. 1993, McLachlan et al. 1995), although it was assumed that these arose from difficulties with the LC method used. However, as reported below, we have now determined that such results are artifacts of sample preparation and enzyme action.

The positive ion-spray LC-MS analysis of a 70-day-old culture sample (Fig. 3a) extracted using Method 1 showed the presence of OA (peak 1), DTX1 (peak 2) and OA diol-ester (peak 5). The diol-ester of DTX1 was not

observed at significant levels in this particular isolate, although we have observed it in other P. *lima* isolates (unpublished results). At first, it was considered that the non-reproducible results were due to cells not being adequately ruptured by sonication, but repeated extraction of sonicated cells resulted in no further extraction of toxins.

Cells could be rapidly and completely disrupted by passing them through a French press, and subsequent methanolic extraction showed increased levels of OA, DTX1 and OA diol-ester (Fig. 3b). If the disrupted cells were held at room temperature for several h, however, the diol-ester concentration decreased while that of OA increased. Similar results were observed if a cell pellet was resuspended in TrisHCl buffer, frozen in liquid nitrogen and allowed to thaw. Fig. 3c shows the results after freezing and then incubating at room temperature for 24 h. All of the diol-ester had disappeared, leaving only OA and DTX1. These observations suggested enzymatic hydrolysis reactions.

A peak at 5.3 min in the m/z 819.5 mass chromatogram (peak 1 m, Fig. 3a) was identified as being due to the methyl ester of OA. The level of this compound was highly variable even between replicate subsamples of culture, and was sometimes greater than that of OA. The amount of methanol used in the extraction step had a marked effect, with lower percentages increasing the level of methyl ester, opposite to what would be expected if there was a problem with extraction yields due to lipophilicity. This suggested a reaction between methanol and the analytes. Different amounts of water in cell pellets during extractions could partially explain the variations in methyl ester levels observed previously. Substitution of deuterated methanol (CD_3OH) in the extraction protocol showed a shift of the m/z 819.5 ion to m/z 822.5, proving that the methyl ester was an artifact of the extraction procedure. This conclusion was substantiated by substitution of acetonitrile or tetrahydofuran for methanol, with elimination of methyl ester formation. However, yields of OA and its diol-ester proved much lower with these solvents for reasons which became apparent after the discovery of DTX4 (see below). When methanol was added to the French press homogenates and the mixtures allowed to incubate, methyl ester could be detected. At 40% methanol, nearly complete conversion of compound 5 to OA methyl ester occurred. This observation, along with the lack of conversion in boiled extracts (Fig. 3d), led to the conclusion that hydrolysis and methanolysis of 5 in these extracts were occurring enzymatically. Immersing the cell pellet in boiling water for 3 min prior to extraction with methanol eliminated the methyl ester formation, but under these conditions the diol-ester concentration was very low (Fig. 3d).

At this point, information on the existence of DTX4 became available (Hu *et al.* 1995). An additional peak close

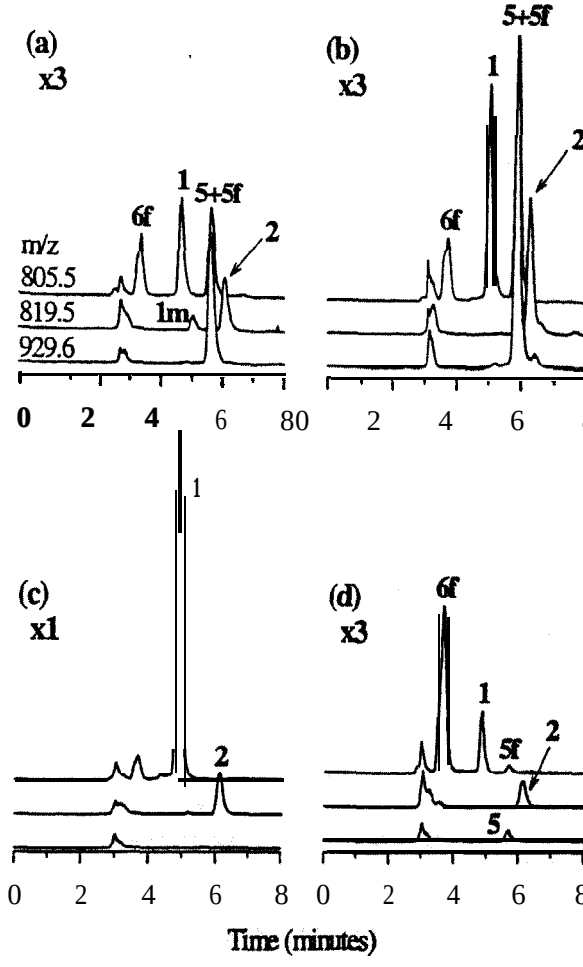


Fig. 3. LC-MS analyses of replicate subsamples of P. lima *culture extracted according to methods 1 to 4 (a to d, in that order). Conditions: positive ion-spray, selected ion monitoring, Vydac 201TP52 column, and 0.2 ml/min aqueous 80% methanol with 0.1% TFA. Peak identities as in Figure 1; 1m = OA methyl ester: f = fragment ion*

to the solvent front in the m/z 805.5 chromatogram (peak 6f, Fig. 3) could then be explained, It is now known that this signal is due to a weak positive fragment ion from DTX4. The peak is most intense in the boiled cell extract. A specific LC-MS method for the analysis of DTX4 has now been developed. Fig. 4 shows the negative ion full-scan LC-MS analysis of the same extract of boiled cells analyzed in Fig. 3d. DTX4 was easily detected by $[M-3H]^{-3}$ and $[M-2H]^{-2}$ ions. Interestingly, a number of related new compounds were also detected. The signals from these compounds are evident in the other reconstructed mass chromatograms in Fig. 4 and peak identities are provided in Table 1. Most of the structural variations appear to be associated with the sulfated end group as only one diol-ester of OA is observed in this *P. lima* isolate, Some derivatives of DTX1 are also observed but at much lower concentrations than those of OA. OA and DTX1 can also

Table 1. DSP toxins observed in *Prorocentrum lima* extracts.

Gpd. #	Mol. wt.	Ions observed (m/z)[a]	Toxin assignment[b]
1	804.5	805.5 (=); 803.5 (-)	Okadaic acid (OA)
2	818.5	819.5 (=); 817.5 (-)	DTX1
5	928.6	929.6, 805.5 (+)	OA diol ester
6	1472.6	489.9, 735.3, 803.5 (-)	DTX4
7[c]	1486.4	494.5, 742.3, 8 17.5 (-)	DTX4 + CH,
8	1488.6	495.2, 743.3, 803.5 (-)	DTX4 + O
9	1504.6	500.5, 751.3, 803.5 (-)	DTX4 + 2 O
10	1514.6	503.9, 756.3, 803.5 (-)	DTX4 + 42
11[c]	1518.6	505.2, 758.3, 817.5 (-)	DTX4 + CH_2 + 2O
12	1552.6	387.2, 516.5, 775.3, 803.5 (-)	DTX4 + SO_3
13	1568.6	391.2, 521.9, 783.3, 803.5 (-)	DTX4 + SO, + O
14	1576.6	524.5, 787.3, 803.5 (-)	DTX4 + 104
15	1584.6	395.2, 527.2, 791.3, 803.5 (-)	DTX4 + SO_3 + 20
16[c]	1598.6	398.7, 531.9, 798.3, 817.5 (-)	DTX4 + CH_2 + SO_3 + 2 O

[a] Positive or negative ironization indicated by (+) or (-) following ions.
[b] Structures of compounds 1, 2, 5 and 6 are given in Figure 1.
[c] Compounds 7, 11 and 16 are derivatives of DTX1 (methyl group at C_{35}).

be determined in the same analysis, although at lower sensitivity than for DTX4. An LC-UV method for detecting DTX4 and the diol-ester was also developed which allowed for the rapid quantitation of these compounds in algal extracts,

Analyses revealed that DTX4 was the most abundant of the DSP toxins in boiled *P. lima* cells, yet was present at very low concentrations in fresh French press extracts. A corresponding increase in diol-ester concentrations in the French press homogenates (Pig. 3b) suggested that enzymatic conversion of DTX4 to diol-ester was occurring within min. Freeze/thaw treated samples also had low DTX4 levels in contrast to those in boiled extracts, providing further evidence for enzymatic hydrolysis of DTX4. It was observed that the cells store most of the DSP toxins in the DTX4 form which is first enzymatically hydrolyzed to the diol-ester when ceils are disrupted and then mom slowly hydrolyzed to OA. From these observations, it appears that DTX4 and the enzyme(s) responsible for its hydrolysis are sequestered in different compartments in the ceil.

The ability to analyze selectively for the suite of DSP toxins and restrict enzymatic hydrolysis using the boiling technique was applied to study the kinetics of DSP toxin production and excretion by *P. lima* in culture. Analysis of independent batch cultures over a 90-day period revealed clear and consistent trends in the intracellular levels of all the toxins (Fig. 5). Total intracellular toxin levels in culture increased linearly with time throughout the culture period. On a per cell basis, a positive correlation with growth rate is indicated. DSP toxin production is therefore not disassociated from growth, as is the case with many

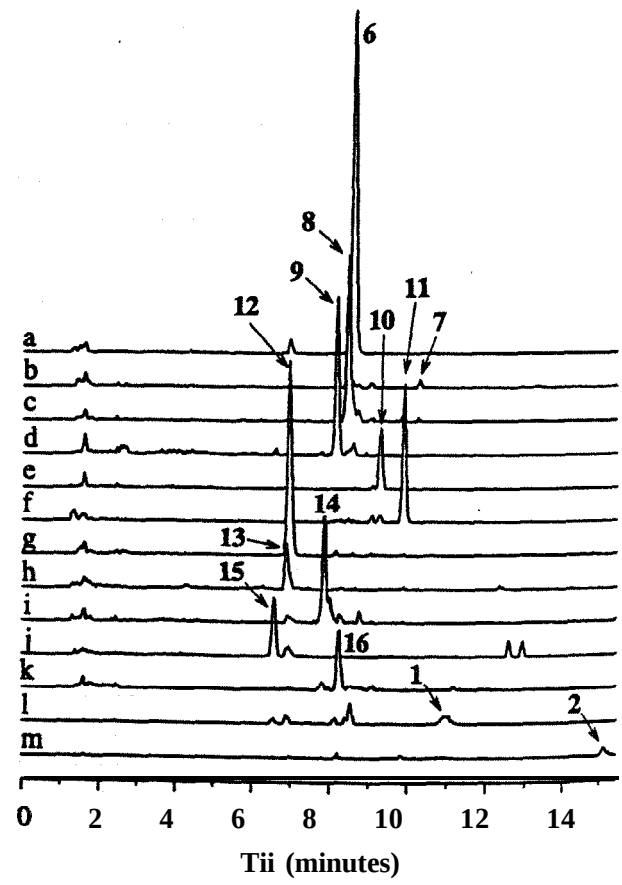


Fig. 4. Negative ion-spray LC-MS analysis of the same P. lima *extract analyzed in Figure 3d using selected ion monitoring of $[M-3H]^{-3}$ and $[M-2H]^{-2}$ ions for traces a-k, and $[M-H]^{-1}$ ion for traces l-m. Peak identities and ions are listed in Table 1.*

secondary metabolites. After cessation of cell growth, toxin production continued, resulting in progressively higher intracellular levels, reaching 40 fmole/cell after 90 days. DTX4 is clearly the dominant intracellular DSP component, exceeding the levels of all other toxins combined. Both OA and DTX1 were present in about equal amounts; however, only trace levels of OA diol-ester were present. OA and, to a much lesser extent, DTX1 were found to accumulate in the medium while only trace levels of DTX4 and the diol-ester were detected (Fig. 6). Release of OA into the medium is probably enhanced by the high aqueous solubility of DTX4 and mediated by the enzymatic pathway described above. This could explain why DTX1 is present at much lower concentrations in the medium than OA even though the intracellular concentrations were similar. Evidence for the role of the hydrolytic enzymes in OA excretion is suggested by the lack of other DSP compounds in the medium and by the rapid conversion of DTX4 to OA-diol-ester upon cell disruption, followed by enzymatic conversion of the diol-ester to OA. Since OA is a potent inhibitor of eukaryotic phosphatases, the excretion of DTX-4, and thus ultimately OA, by *P. lima* may constitute a chemical defense system directed at predators, competitors, or pathogens (Windust et *al.* in press).

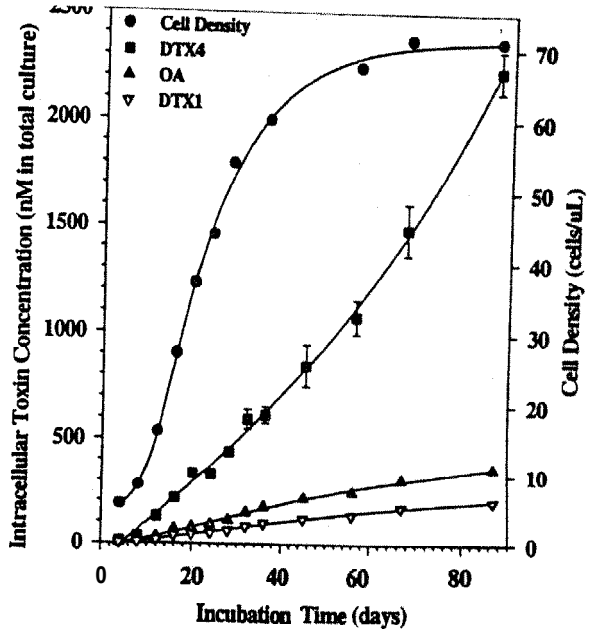


Fig. 5. Cell growth and intracellular toxin *production by* p. lima *in batch culture.*

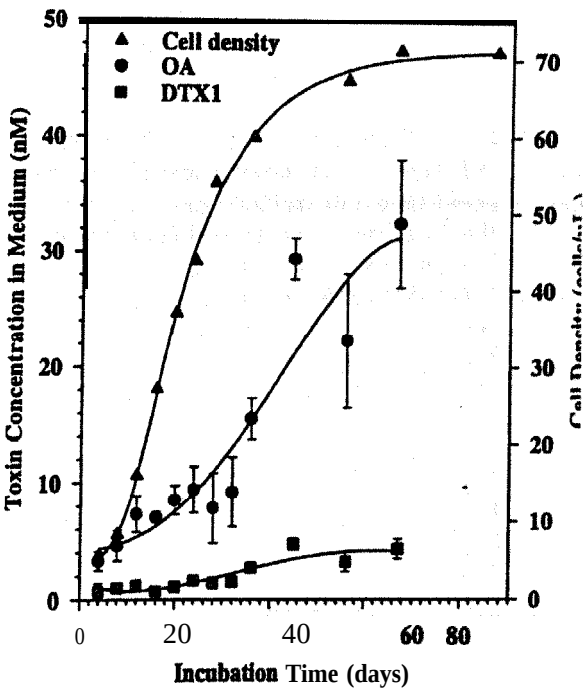


Fig. 6. Cell growth and excretion of toxin to *medium by* P. lima *in batch culture.*

CONCLUSIONS

We are currently investigating the fate of the diol-ester and DTX4 derivatives in shellfish. We hypothesize that they are hydrolyzed to OA and DTX1 due to esterases in the shellfish digestive gland as well as those from the algae. Although the diol-ester is not toxic, hydrolysis yields OA. Therefore, comprehensive analysis of all toxin-related compounds is necessary to properly assess the toxicity of any new plankton isolate. Clearly LC-MS will be an important tool for this task. The fingerprinting of the suite of toxins is also of interest for chemotaxonomic studies. In preliminary experiments, we have seen tremendous variations in toxin profiles among different isolates of *P. lima* and between different species of Prorocentrum. For those laboratories without access to LC-MS equipment. the freeze/thaw/hydrolyze method should be useful for assessing the toxic potential of plankton samples and culture material. Experiments directed toward the accumulation and depuration of toxins in shellfish using *P. lima* should be carefully designed to avoid the analytical errors that are possible due to enzymatic transformations.

ACKNOWLEDGMENTS

We thank W.R. Hardstaff and A. Reeves for technical assistance and J.L.C. Wright for a sample of DTX4. This research was supported in part by DFO/NSERC subvention and NSERC operating grants awarded to JLM. NRCC No. 39692. /

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Osteological Evaluation in Artificial Seedlings of *Paralichthys olivaceus* (Temminck and Schlegel)

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ABSTRACT

As one of the approaches to clarify the morphological characteristics in artificial seedlings, the axial skeleton of the Japanese flounder, *Paralichthys olivaceus* (Temminck and Schlegel), was observed. Basically, the axial skeleton of *P. olivaceus* is composed of 38 total vertebrae, viz, 11 abdominal and 27 caudal vertebrae. However, in artificial seedlings, the number of total vertebrae was fewer than that in wild fish, and the constitution of vertebrae was also more variable (mean ± SD = 36.4 ± 2.5). The frequency of central fusion was 63% in abdominal and 74% in caudal vertebrae in artificial seedlings, while it was 0% in abdominal and 4% in caudal vertebrae in wild fish. In addition, each symptom of fusion in artificial seedlings was generally serious. Concerning the frequency of central fusion in artificial seedlings, there was approximately one gentle peak in abdominal vertebrae and three peaks in caudal. The completion of central development was me fastest in the basioccipital articulatory process, the second in the urostyle, while the last in the 25th or 26th vertebrae where a relationship between the frequency of fusion and the velocity of ossification was suggested.

INTRODUCTION

In the last two decades, the technique of seedling production has been remarkably improved in Japan. In some important commercial fish species, the mass production of artificial seedlings has become possible. However, in artificial seedlings, morphological malformation constantly appears at high frequency and the quality of seedlings is not at the level of wild fish. The incompleteness of feeding and swimming organs in early development is said to affect the survival rate greatly after the juvenile period, which is an emergent problem to be solved in the production of healthy artificial seedlings. This study examines the developmental changes in the trunk and clarifies the morphological characteristics of artificial seedlings.

The Japanese flounder, *Paralichthys olivaceus* (Temminck and Schlegel), is one of the typical metamorphosing fishes in Japan. In order to elucidate the relationship between osteological development and bone anomaly, some standard structures in the skeletal system of *P. olivaceus* were reported (Hosoya and Kawamura 1993). In this paper, using an axial skeleton which supports the body, the osteological characteristic of artificial seedlings is clarified under comparison with wild fish. That is, the standard structure of an axial skeleton and a bone anomaly typically seen in artificial seedlings are described. Then, from the point of view in osteological development, the factors causing bone anomaly are assumed. Finally, including the information about morphometry, the developmental stages in *P. olivaceus are* rearranged and the relationship between character expression and morphogenesis is discussed as well.

MATERIALS AND METHODS

Artificial seedlings of *P. olivaceus* were reared and fed with a series of rotifer, atremia and pellets from February to June in 1994 and 1995. Rotifers had beforehand been enriched with a phytoplankton, *Nannochloropsis oculata*. Rearing experiments were done in a 500 L fish tank at unadjusted water temperature of 15 to 19°C. Seedlings were first reared in stationary water for a week and then transferred to running water (50 L/h). Sampling was done twice a week. Collected samples were immediately fixed with 3% formalin, 70% ethanol and Bouin solution, respectively. As a control, wild fish were sampled from wild populations in the following areas: the Tone River, Maizuru, Kasumi, Aoya, Tenzin, the Yura River, Kazusa and Tsushima (Fig. 1). The skeletal system was stained with either a double-staining technique (Kawamura and Hosoya 1991) or a single Staining technique, using only alizarin red S or alcian blue, and examined under a binocular microscope. Ossification was histologically confirmed with the appearance of osteocytes. As for anatomical terminology relating to the skeletal system, Hosoya (1991) was followed. In counting vertebrae, abdominal

and caudal vertebrae were defined as vertebrae equipped with parapophyses or a sharp haemal spine, respectively (Fig. 2). The urostyle was included in the caudal vertebrae.

RESULTS

CHARACTERISTICS OF VERTEBRAL COLUMN IN *P. OLIVACEUS*

To elucidate the morphological characteristics of artificial seedlings with bone anomalies, it is necessary to utilize the accurate information on the skeletal system of healthy wild fish from wild populations. Though *P. olivaceus* is a commercially important fish species in Japan, the information about its skeletal system is scarce except for the conclusive descriptions about flatfishes by Amaoka (1969) and Sakamoto (1984). The standard structure and the developmental process of the vertebral column in *P. olivaceus* are described below.

GENERAL MORPHOLOGY

Abdominal vertebrae are expanded up and down. The basic number of abdominal centra supporting the vertebrae is nine. The first abdominal vertebra is articulated with the basioccipital articulatory process (BOAP) at the anterior tip. Each abdominal vertebra is equipped with a neural spine dorsally and a neural arch ventrally, for protecting the notochord (Fig. 2). On the fifth abdominal vertebra or the posterior element, a pair of parapophyses develop ventrally. The first to fourth neural spines are remarkably thick compared with the others, which represents an important characteristic of the vertebral column in *P. olivaceus* (Fig. 5, upper). Left parapophyses are constantly longer than right ones, probably reflecting an asymmetrical body shape. On the distal end of a parapophysis, a rib is attached. The caudal vertebrae are composed of approximately 27 centrums. At the posterior end, they are transformed into the complicated urostyle. Each caudal vertebra is equipped with a pair of long haemal spines ventrally, which form a haemal arch at the base. The first haemal spine is thick and extends ventrally. On its anterior surface, there is a groove receiving the first proximal pterygiophore from the anal fin.

DEVELOPMENTS OF VERTEBRAL COLUMN

The developmental process of the vertebral column in *P. olivaceus* is described below (Figs. 3.4) in stages defined by Minami (1982).

Stage A. The skeletal system is underdeveloped. Osteocytes surrounding the notochord are not recognized at all. In the neurocranium, one of the components in the axial skeleton, trabecula cartilages, was stained dark blue with alcian blue.

Stage B. The segmentation of the notochord began in part. As the first bone elements, the first and second neural

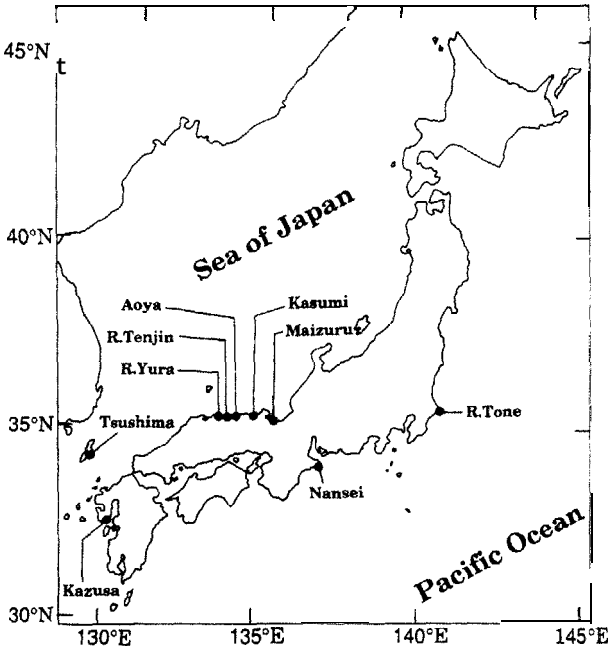

Fig. 1. Sampling sites of Japanese flounder, Paralichthys olivaceus.

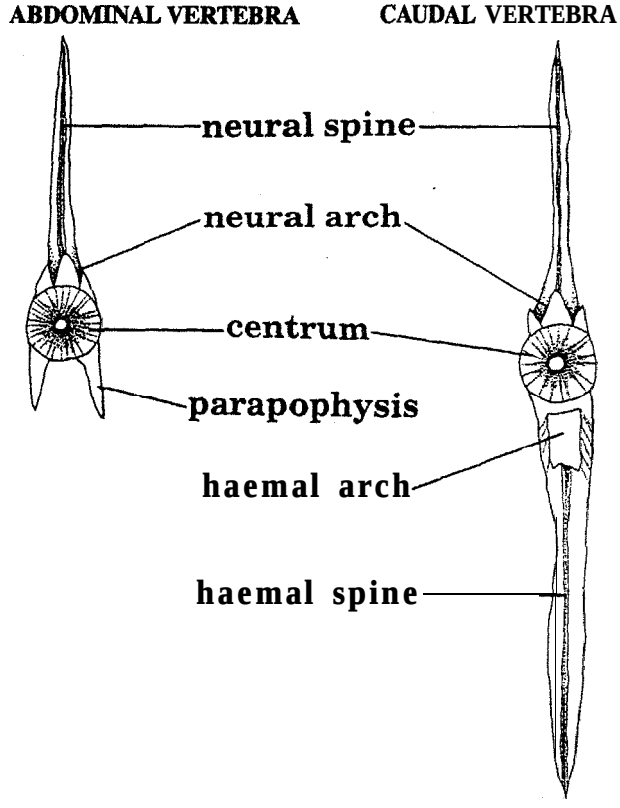

Fig. 2. Skeletal structures of typical abdominal and caudal vertebrae in *frontal view.*

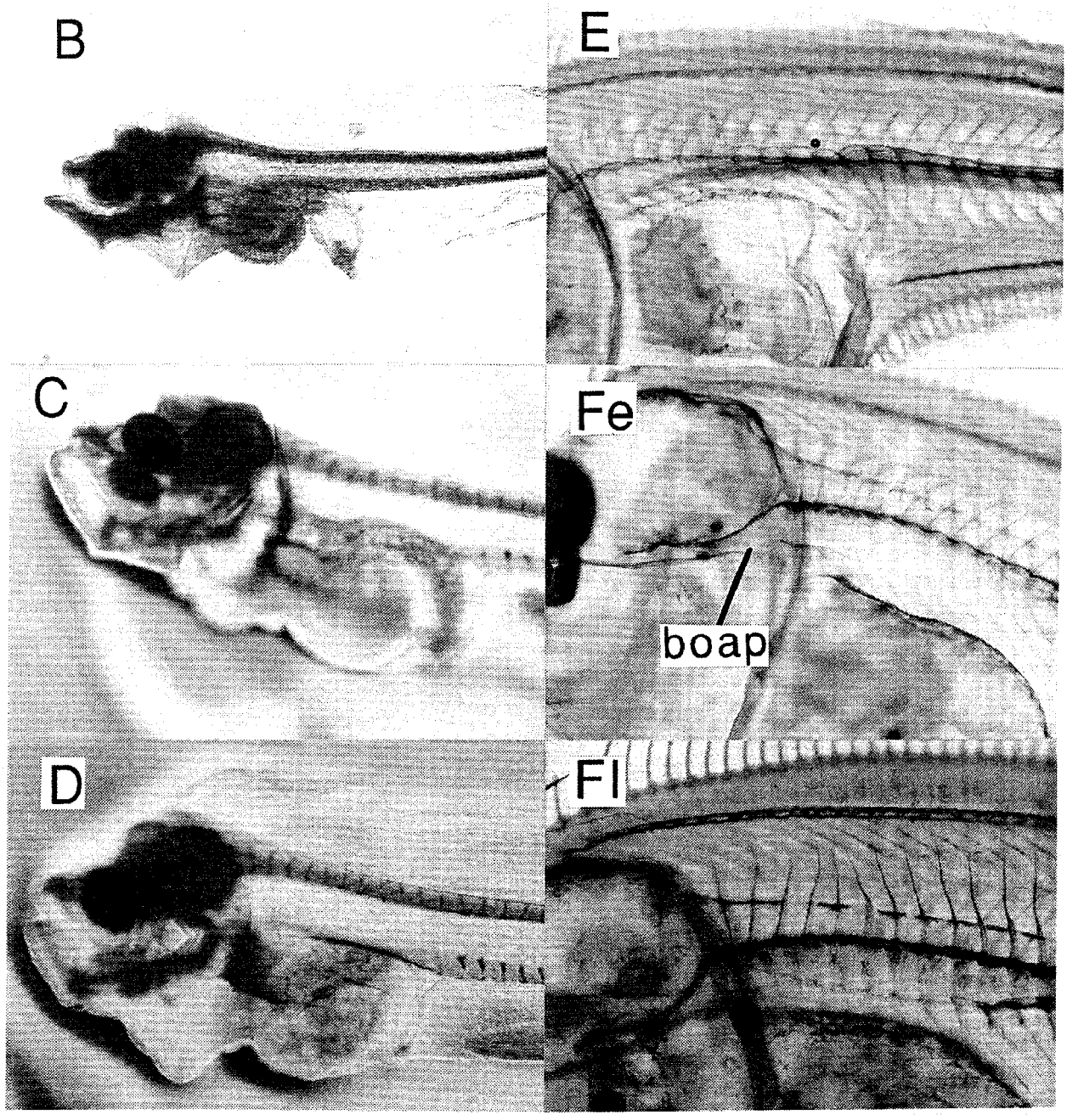

Fig. 3. Osteological development of abdominal vertebrae in Paralichthys olivaceus. *Each character corresponds to a developmental stage as defined by Minami (1982) except for F, which here divided into early (Fe) and late (Fl) substages.*

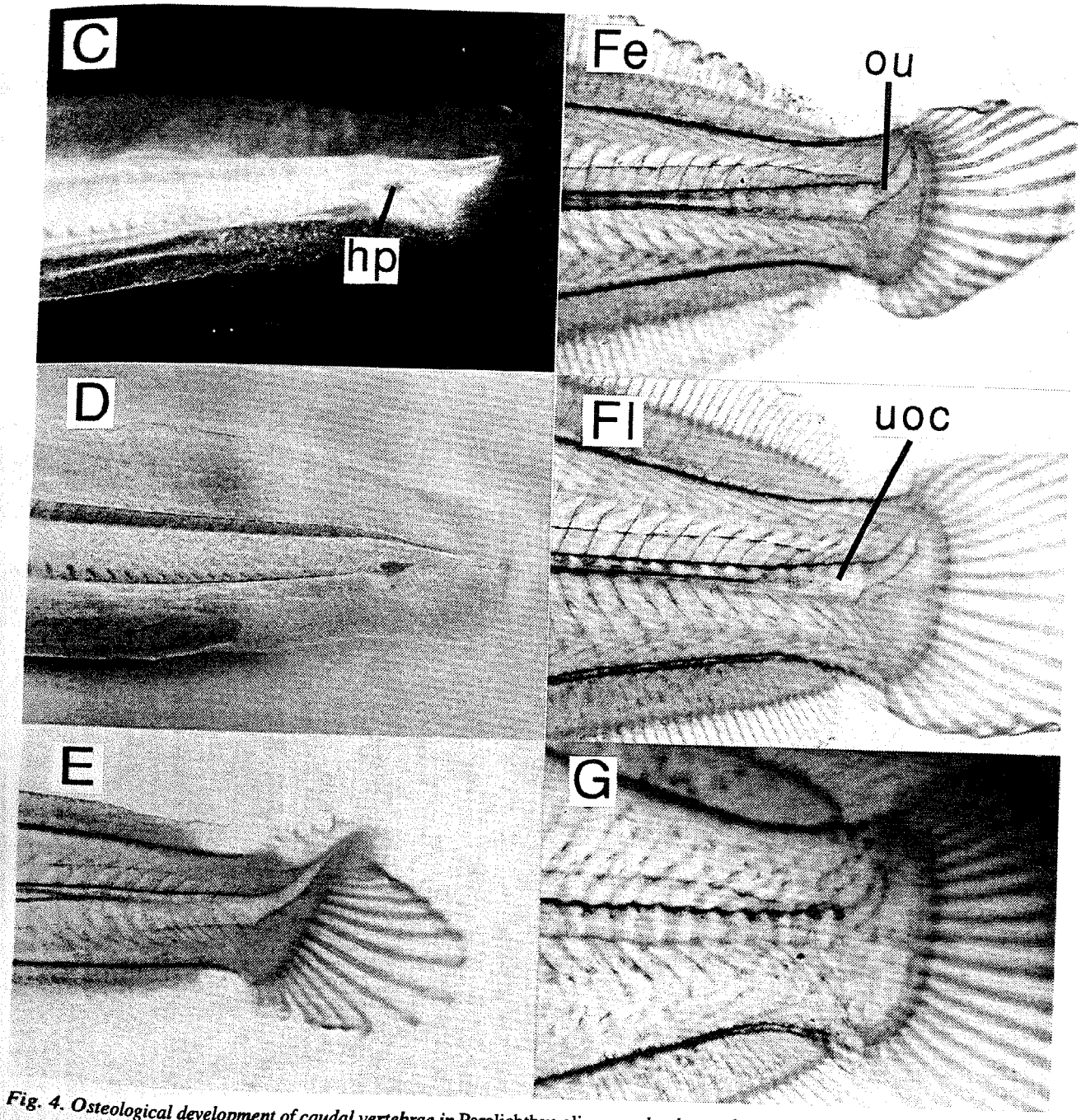

Fig. 4. Osteological development of caudal vertebrae in Paralichthys olivaceus. ***hp, hypural primordium; ou, ossified urostyle; uoc, unossified centrum.***

arch cartilages appeared. In the neurocranium, cartilages surrounding the otic capsule were apparent. Trabecula cartilages expanded much further.

Stage C. This stage is the first important phase in the development of the vertebral column. Though almost all neural arch cartilages appeared, the arrangement of neural arches was not serial, because the fifth to seventh neural arch cartilages from the posterior end had not yet been formed. Likewise, in the haemal arch series, all cartilage elements appeared. In the lower caudal region, two large cartilage masses, hypural primordia, were also apparent. Parapohyses and haemal spines were totally underdeveloped. However, the first haemal spine, the longest one located at the anterior end of the vertebral column, was exceptionally stained dark blue with alcian blue. As elements relating to the vertebral column, 10 proximal pterygiophores were recognized at the base of the long dorsal fin rays. With the second to fourth proximal pterygiophores, median pterygiophore cartilages were already articulated.

Stage D. The notochord is straight; however, its posterior end began to bend dorsad. Hypural and epural primordia also appeared ventrally just behind the posterior end of the notochord.

Stage E. Tendency of dorsal bending in the notochord has become more apparent. All the parapophyses and the second and haemal spines posterior to the second appeared. All the cartilage elements constituting the vertebral column were present. In the neurocranium, the bottom and frontal regions began to be ossified.

Stage F. This stage is the second important phase in development of a vertebral column, viz, ossification drastically proceeds. Concerning cartilage bones, the first to 20th neural spines and the first to 10th haemal spines were stained red with alizarin red S. The degree to be stained was remarkable especially in the first to fourth neural spines and the first to seventh haemal spines. In adult fish, neural arches, neural spines and haemal spines, except the posterior ones in the caudal region, were stained dark red. All the parapophyses were ossified. On the other hand, concerning membrane bones, BOAP was the first to be ossified (Fig. 3), and the urostyle the second (Fig. 4). In addition, each centrum began to be ossified. The ossification degree in the centrum had progressed greatly in the anterior elements of the abdominal vertebrae, and in the seventh to 1 lth elements of the caudal vertebrae. Contrary to this, the 23rd to 26th caudal vertebrae (the fifth to second preurostyles), in which ossification was strikingly late, formed characteristic unossified centrums (UOC) between the preceding caudal vertebrae and theurostyle (Fig. 4).

Stage G. Each centrum stands together almost in a straight line with a break point identical with the 25th to 26th caudal vertebrae (the third to second preurostyles). Between the neighboring caudal vertebrae, however, a gap of about one third or half width of a centrum was present. The neurocranium began to be transformed. The frontals, the parietals, the sphenotics, the pterotics and the supraoccipitals were recognized, respectively, in it. Each proximal pterygiophore cartilage of the anal fin, articulated with the first haemal spine, was stained dark blue with alcian blue.

Stage H. The skeletal system of the vertebral column was almost completed by the connection of the 25th to 26th caudal vertebrae with the other ossified vertebrae. However, centrums only surrounding the notochord had not yet been constricted. Many pterygiophores in dorsal and anal fins still remained cartilaginous.

Stage I. Pterygiophores in dorsal and anal fins begin to be ossified and face either corresponding neural or haemal spine originating from a centrum.

COMPARISON OF ARTIFICIAL SEEDLINGS WITH WILD FISH

Meristic counts are known to be strongly affected by environmental factors like temperature during development. From hatching, artificial seedlings are subjected to an environment strikingly different from what they naturally experience. So, it is likely that meristic counts in artificial seedlings are different from those in wild fish. In the following, to determine the number of vertebrae which is a typical meristic count, the frequency of shrinkage and fusion in the centrum of artificial seedlings was compared with wild fish from some localities in Japan.

NUMBER OF VERTEBRAE

In wild fish, the mean of abdominal vertebrae was 11.0, except for a few samples from Maizuru, Kyoto (Table 1). The standard deviation (SD) fluctuated (0.2 in the Yura River, Tottori, 0.3 in Kasumi, Hyogo, 0.6 in Maizuru), while it was stable (0.0 in the other localities). On the other hand, in artificial seedlings, the mean was 10.9 and SD 0.6. Even in Kazumi, where the sample number was the same in both groups, SD was larger in artificial seedlings (0.6) than in wild fish (0.3). This means that the variation of abdominal vertebrae is larger in artificial seedlings. Accordingly, in artificial seedlings, the number of abdominal vertebrae is smaller and more variable than in wild fish.

The number of caudal vertebrae in wild fish ranged between 26.7 and 27.6 in the mean. The SD converged between 0.4 and 0.6, in spite of the differences of both localities and the number of samples. On the other hand, in artificial seedlings, the mean was 25.5 and SD 2.5. In artificial seedlings, the number of caudal vertebrae was two smaller and more variable than in wild fish.

The number of total vertebrae in wild fish ranged be-

tween 37.3 and 38.7 in the mean, and between 0.4 to 0.6 in SD. In artificial seedlings, it was 36.4 in the mean and 2.5 in SD. In comparison of artificial seedlings with wild fish, the counts in the vertebrae were the same as those of the caudal vertebrae. Accordingly, the differences between wild fish and artificial seedlings in the mean and SD of the total number of vertebrae are considered to reflect directly to those of the caudal vertebrae.

SHRINKAGE AND FUSION IN CENTRA

The most typical symptom in bone anomaly observed in the vertebral column of ***P. olivaceus*** is that the neighboring centrums shrink and fuse each other (Fig. 5, middle, lower). In the artificial seedlings from Mie, the frequency of shrinkage and fusion in the centrum amounted to 63% (32 in 5 1 individuals). It showed a gentle peak around the fourth to seventh abdominal vertebra, while it was the lowest between the BOAP and the first abdominal vertebrae, at the anterior end of the vertebral column (Fig. 6). On the other hand, in wild fish from Tottori, shrinkage and fusion of abdominal vertebrae were not seen in any of 221 individuals.

The frequency of shrinkage and fusion in caudal vertebrae amounted to 74% (37 in 50 individuals) in the artificial seedlings from Mie. On the whole, the frequency presented three peaks: the gentle highest peak around the 10th to 12th caudal vertebrae, the second peak around the 18th to 19th and the third small peak around the 24th to 26th (Fig. 7). No fusion was seen between the 26th and 27th caudal (the urostyle) vertebrae, the fastest to be ossified. On the other hand, in the wild fish from Tottori, the frequency of fusion was only 4% (8 in 221 individuals). Though fusion was seen to lie scattered in the posterior caudal vertebrae, it composed a characteristic peak around the 25th and 26th caudal vertebrae. This peak seemed to correspond to the third peak seen in wild fish.

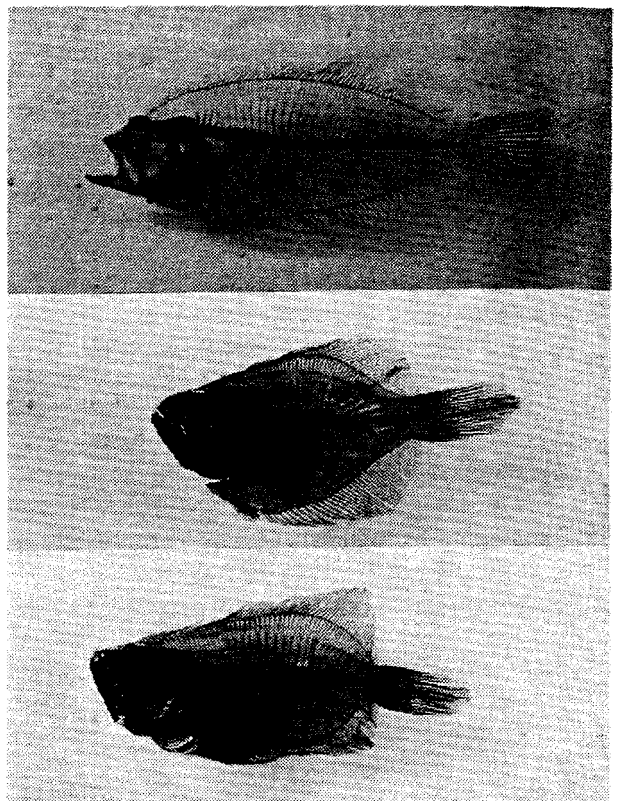

Fig. 5. Body shortenings due to central fusions in reared Paralichthys ***olivaceus. From top to bottom, normal, abdominal fusions, and caudal fusions.***

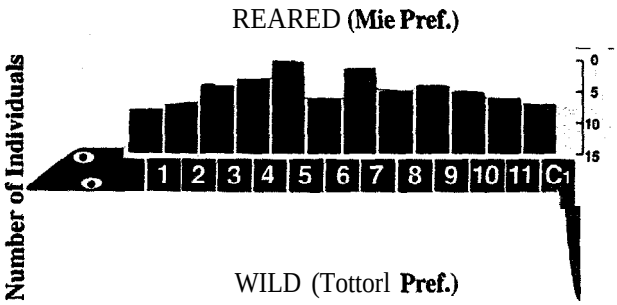

Fig. 6. **Central** ***fusions in abdominal vertebrae of*** Paralichthys **olivaceus.**

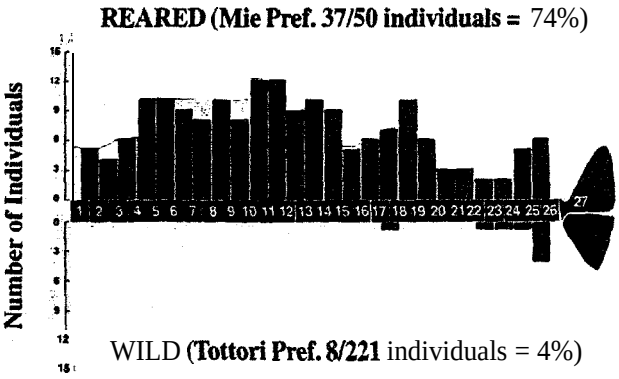

Fig. 7. Central fusions in caudal vertebrae of Paralichthys olivaceus.

DISCUSSION

DEVELOPMENTAL STAGE

The developmental process of the axial skeleton in ***P. olivaceus*** was described following the developmental stages defined by Minami (1982). The formation of vertebrae proceeds intermittently from stage A, a stage soon after hatching, to stage I, a stage soon after settlement. In the formation, two remarkable phases, when ossification drastically proceeds, were also recognized, That is, stages C and F. The former is characterized by the formation of cartilages, such as neural and haemal arches. The latter is a phase when ossification drastically proceeds, regardless of origin. Accordingly, from the point of view in the developmental process of the vertebral column, the developmental stages of ***P. olivaceus*** were grouped into three Periods, that is, A-B, C-E and F-I.

The gradation in early development of *P. olivaceus was* already ascertained with morphometric analysis, in which two points of drastic changes were found around 7 nun and 10 nun TL, respectively. The first point was situated between *stages* D and E, while the second between stages F and G. Paying attention to changes in external organs, the developmental process of *P. olivaceus* is to be grouped into three periods: A-D, E-F and G-I. Comparing the result in morphometry with that in development of the vertebral column, the staging does not always agree with the staging based upon changes in external organs. The shift of one developmental stage to the next always occurs earlier in the former stage than in the latter. However, it is obvious that changes in external organs, reflected in morphometry, follow those in internal organs, e.g., the skeletal system. The structural changes in vertebrae supporting the body bring about the malformation of the external features. The points of changes in the early life history of P. *olivaceus,* especially feeding habit, are in accord with the staging based upon external organs (Hosoya 1991). Accordingly, the precedence of bone formation in the vertebral column to the formation of external organs might be regarded as a preadaptation to the upcoming stage.

Concerning cranial and caudal skeletons, in both skeletons, there is a discontinuous zone characterized by drastic ossification of membrane bone between stages D and E. Contrary to this, in the vertebral column, a drastic phase of ossification was present at stage F. Though it is difficult to explain the difference of phases in ossification between cranial and caudal skeletons, and vertebral column, it is partly explained by the functional property in each bone element. In bone formation of *Pagrus major,* the life-support or feeding organ, swimming organ, **and body-support** organ were formed in order (Matsuoka 1987, Hosoya 1991). The cranial skeleton involves the basic bone elements related to life-support and feeding organs, e.g., gill arches and a gill apparatus, while the caudal skeleton is an important swimming organ, in charge of propulsion. It seems common in not only *P.major* but also the other marine fishes with larval and juvenile stages, that the formation of body-support organs is later than that of the cranial and caudal skeletons.

CHARACTERISTICS OF ARTIFICIAL SEEDLINGS

As one of the approaches to clarify the morphological characteristics of artificial seedlings, both wild fish and artificial seedlings of *P. olivaceus were* compared, concerning the frequency of centrum fusion. As a result, in wild fish, fusion was hardly seen in other vertebrae, except for the 25th and 26th vertebrae. Contrary to this, in artificial seedlings, fusion was frequently seen. Especially in the seedlings from Mie, the frequency of fish with any fusion in the centrum was 74%. As the same tendency was also seen in the seedlings from Tottori, centrum fusion may be said to be characteristic of artificial seedlings. In fact, when the *stomach* contents of a paddle crab, *Ovalipes punctatus,* captured during research on predators of released *P.* olivaceus by the Tottori Prefectural Fisheries Experimental Station were analyzed, both fused abdominal and multi-fused vertebrae, not seen in wild fish, were observed together with such bone splinters as the urohyal, a lower jaw, and the hypurals, which certainly represent taxonomic characters of *P. olivaceus.* This evidence of predation on P. *olivaceus* with centrum fusion suggests that centrum fusion is a problem to be overcome in artificial production of healthy seedlings, which may also affect seedling survival upon release. Since centrum fusion is not rare in wild larvae, elimination of such larvae by intense natural selection absent in hatcheries must occur. If artificial seedlings with centrum fusion will be rapidly eliminated by natural selection upon release, then resource enhancement effects from release of such seedlings will be overestimated. Then, it becomes absolutely necessary to estimate the post-release survival rate of individuals with centrum fusion empirically. As one of the practical methods for this purpose, in closed environments like salt pens, periodical estimation of the frequency of fish with centrum fusion after being released might be considered.

Bone formation and anomaly

Artificial seedlings are characterized by central fusion, whose frequency shows one gentle peak in abdominal vertebrae and three peaks in caudal. The reason why the frequency of central fusion is high in artificial seedlings but low in wild fish is that the breeding environment of artificial seedlings is considered to deviate from the most optimal condition to control the morphogenesis of meristic characters, compared with the natural habitats of *P. olivaceus.* The factors inducing central fusion under the breeding environment are presumed to be: shortage of nutrient substance, water temperature, concentration Of Ca^{2+}, shortage of vitamin C, parasites, fish density and stress (Kitamura 1969, Matsusato 1986). It is, however, difficult to specify the direct factors. Interestingly, among the peaks in the frequency of central fusion in artificial seedlings, only *the* third peak in the caudal vertebrae was seen in wild fish as well. This peak is present around the 25th and 26th caudal vertebrae, which are final formations in *P. olivaceus.* Though in the formation of axial skeleton, the genetically programmed fate in development might be considered to proceed, adjusted by environmental factors, the 25th and 26th caudal vertebrae are inferred to be the most sensitive region in development of *P. olivaceus,* in both artificial seedlings and wild fish. Body shrinkage is induced by either central shrinkage or shortage of an in-

terval between the neighboring centrums (Matsusato 1986). As each neighboring centrum is linked by a tendon, the 25th and 26th caudal vertebrae might be considered to be drawn closer and fused by the shrinkage of tendons before the completion of centrum ossification.

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Further Advances Toward the Microbial Management in Closed Recirculating Production Systems of Marine Fish Larvae

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ABSTRACT

Management of the microbial species composition in closed culture systems is a viable strategy to enhance production of marine fish larvae. The procedure consists of eliminating the major sources of microbes through specific disinfection techniques and replacing the unwanted bacteria with a beneficial microflora. Disinfection methods were chosen to eliminate unwanted toxic residues and selection for resistance in the microflora. Selection of beneficial bacteria for larval culture was carried out as a two step procedure: (1) determining active nitrifying bacteria. and (2) evaluating either commercially available bacterial supplements or single bacterial strains found beneficial for the culture of diverse marine organisms, each supplied with the best nitrifiers as previously determined. Results to date are encouraging but one task remains and this is to develop rotifer cultures with known bacterial composition. Experiments with the marine fish *Sciaenops ocellatus* showed the feasibility of this management strategy to enhance fish production.

INTRODUCTION

Technological developments in marine fish culture are moving toward high density production systems. Large variability in larval survival and growth is recurrently observed among tanks stocked with larvae from a single batch of eggs. This variability in larval production needs to be reduced in order to make intensive fish production in closed systems a viable industry. The influence of the microflora on this variability has not been evaluated.

METHODS AND RESULTS

Variables were evaluated independently. However, the most successful method of production as determined at each step was incorporated into the procedure to test subsequent variables in order to build up a management strategy.

ELIMINATION OF BACTERIA OF UNKNOWN CHARACTERISTICS

WATER TREATMENT

Tanks with internal biological filters (Craig et *al.* 1990) were filled with 1.50 L tap water and treated with sodium hypochlorite (10 ppm Cl-, final concentration). Following overnight aeration, tanks were drained rinsed copiously with tap water and filled with either raw seawater (control) or seawater filtered through 0.2 pm industrial cartridges. Seawater was aerated overnight before the addition of larvae.

CULTURE TECHNIQUES

Twelve hr after hatching, fish larvae were transferred to culture tanks at a density of 2,000 tank-t. Three or four replicates were set per treatment. Beginning on the third day of culture, larvae were fed daily on washed rotifers, maintaining a prey density of 1 to 5 ml^{-1}. Artificial feeds were supplied to culture tanks with automatic feeders, at a rate of 0.4 to 1 g $tank^{-1}$ day-'. After 10 days of culture. survival rates were calculated as:

Final number of fish/initial number (2,090) x 100.

Measurements were taken on 50 live, anesthetized fish from each tank, using a stereomicroscope, and a digitizing tablet and Sigma Scan software. Percentage survival and standard length were analyzed using one and two-way ANOVA and Tukey's multiple range test (T-method; Sokal and Rohlf 198 1) to determine the differences between treatments at the 0.05 level of probability. Larval growth was significantly higher in filtered seawater than in raw seawater (Tukey's test, $p < 0.05$; Fig. 1).

EGG DISINFECTION

Fish eggs were treated with 3% hydrogen peroxide to obtain bacteria-free larvae (Douillet and Holt 1994). Eggs in control treatment were exposed to filtered seawater and manipulated as eggs treated with hydrogen peroxide. Larvae hatched from treated or non-treated eggs were cultured in filtered seawater and fed as previously described.

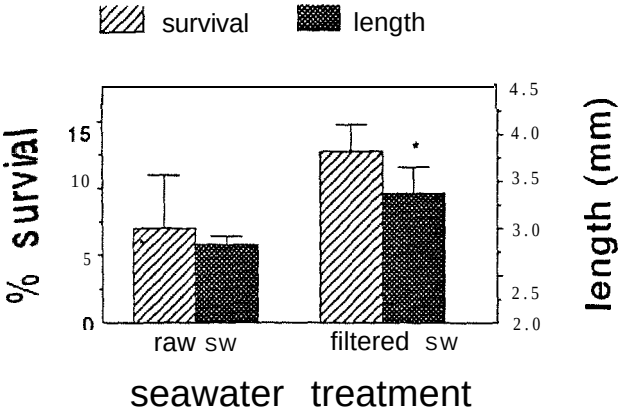

Fig. 1. Survival and growth of red drum larvae after IO days culture on either raw (control) or *0.2 µm filtered seawater Each column is based on three replications. Column with a star is significantly different from control treatment (Tukey's test, p<0.05).*

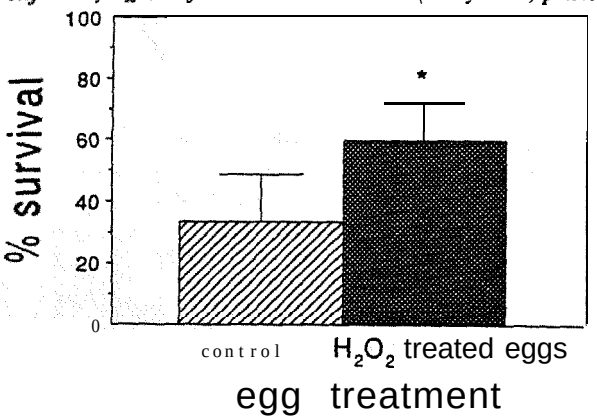

Fig. 2. Survival of red drum larvae after IO days culture following treatment of eggs with hydrogen peroxide at *3% final concentration. Eggs in control treatment were exposed to filtered seawater and manipulated as eggs treated with hydrogen peroxide. Each column is based on 12 replicates (four experimental runs with three replicates each). Column with a star is significantly different from control treatment (Tukey's test, p<0.05).*

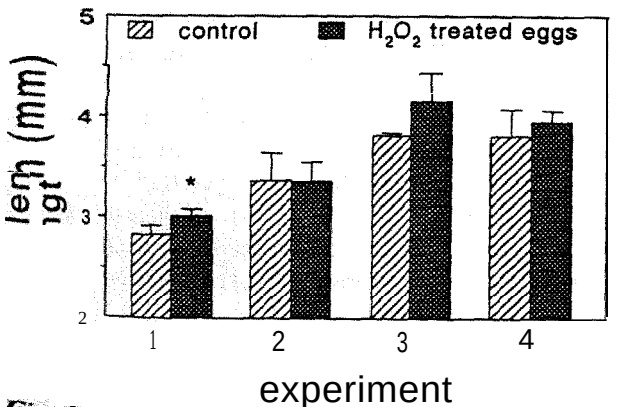

Fig. 3. Length of red drum larvae after 10 days culture following treatment of eggs with hydrogen peroxide at 3% final concentration. Eggs in control treatment were xposed to filtered seawate r *and maniupulated as eggs treated with hydrogen peroxide. Each column is based an 3 replicates. Column with a star is significantly different from its control treatment (Tukey's test, p<0.05).*

The experiment was run four times. Survival data was combined and showed a statistically significant improvement by disinfecting the eggs (Tukey's test, $p < 0.05$; Fig. 2). Growth data was analyzed for each experimental run due to differences between experiments. Larvae from disinfected eggs were significantly larger than larvae from non-treated eggs only in experiment 1 (Tukey's test, $p < 0.05$; Fig. 3).

SELECTION OF BACTERIA BENEFICIAL FOR LARVAL CULTURE

NITRIFYING BACTERIA

Bacteria-free larvae were cultured in filtered seawater and fed as described above. Tanks were covered with individual clear plastic bags to maintain independent microbial communities in each tank. Five different commercially available nitrifying bacterial mixtures were added independently to tanks at 250 ppm on the third day of culture. Control cultures did not receive any addition of nitrifying bacteria. Although no significant differences in larval survival or growth were detected between treatments after 10 days of culture, the rate of nitrification was significantly higher in tanks receiving the addition of nitrifiers "5," a commercial blend of bacteria, than in all the other treatments (Tukey's test, $p < 0.05$; Fig. 4).

BACTERIAL SUPPLEMENTS

Twelve bacteria strains and three commercial microbial additives were tested. Bacteria-free larvae were cultured in filtered seawater and fed as described above. Bacterial additives were added to the tanks as soon as they were filled with filtered seawater on day 1, and on days 4 and 7. Bacteria were added at a final concentration of 1×10^5 cells ml^{-1}. Nitrifiers "5" were added on the third day of culture to all tanks. No bacterial additives besides nitrifiers "5" were added to control cultures. There was an improvement in mean larval survival by the addition of a bacteria strain in experiments 1 and 4 (Fig. 5); however, because of large standard deviations, there was no statistical difference in survival between treatments. In the first three experiments, the addition of the probiotic reduced the coefficients of variation of larval survival between replicates compared to control cultures. In the fourth experiment, addition of the probiotic enhanced larval survival; however, this time the addition of the probiotic increased coefficients of variation of larval survival between replicate cultures, which was not consistent with previous experiments (Table 1).

CONCLUSION

Experiments with the marine fish *Sciaenops ocellatus* demonstrate the feasibility of applying a microbial man-

agement strategy. Complete larval mortalities frequently observed in some tanks prior to the use of microbial management were eliminated by following the methods described here. These microbial management techniques can be easily applied in commercial hatcheries.

The utilization of probiotics did not satisfy the requirement of consistent improvement in larval survival. Rotifers constitute a major source of unwanted bacteria in culture systems. Daily additions of large quantities of unwanted microbes contaminating the rotifers might have added to the variability in fish culture production and reduced the beneficial effects of probiotics which were only added every three days. Research in progress deals with methods of disinfecting and culturing rotifers with selected microbes so that no unwanted bacteria are added to larval culture systems. The integration of this last step will permit a more controlled composition of the microbial community in larval cultures and a more predictable fish production in closed systems.

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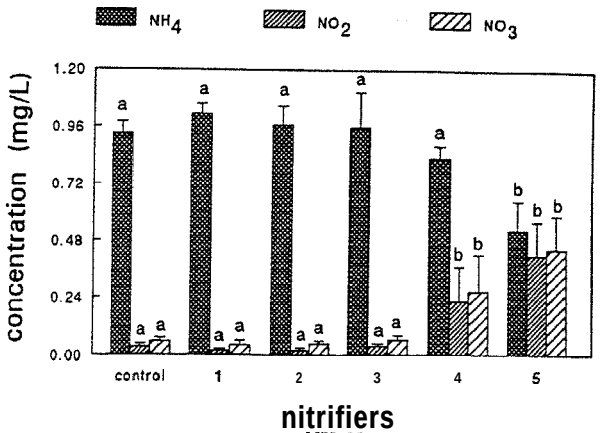

Fig. 4. Final concentrations of NH_4-N, NO_2-N and NO_3-N in mg/L^{-1} in 10-day-old cultures of red drum larvae either inoculated with different nitrifying bacteria or not receiving any addition of bacteria (control). Three replicate tanks were run per treatment. Columns significantly different (Tukey's test, $p<0.05$) from other columns representing same form of nitrogen were identified with different letters.

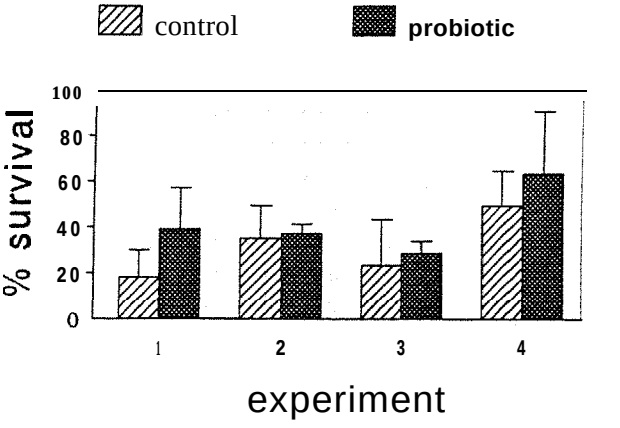

Fig. 5. Survival of red drum larvae after 10 days culture with the addition of a bacterial probiotic. Conttvl cultures did not receive inoculations of the probiotic. Four replicate tanks were run per treatment. Significant differences in survival were determined between experiments (two-way ANOVA, $p<0.05$), but not between tretments in any of the experiments (one-way ANOVA, $p<0.05$).

Table 1. Coefficients of variation of larval fish survival in lo-day-old larval cultures either receiving or not (control) additions of probiotic.

Experiment	Control	Probiotic
1	68.65	48.03
2	42.56	11.11
3	89.23	18.56
4	33.29	46.26

Accumulation and Toxicity of Cadmium in Marine Fish

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ABSTRACT

Salinity affects the toxicity of several heavy metals in the aquatic biota, particularly cadmium. In this study the difference of physiological functions for osmotic regulation between freshwater and saltwater fish was applied to examine cadmium accumulation and its toxicity in marine fish. In the girella exposed to cadmium, the highest concentration of cadmium was found in the liver, followed by the kidney. The cadmium concentration in the gills was low. Cadmium accumulation in the gills of mummichog exposed to cadmium disproportionately decreased with increasing salinity, while the cadmium concentration in the intestine increased with salinity. After an intraperitoneal injection of cadmium bound to metallothionein to mummichog, the cadmium concentration in the kidney decreased with increasing salinity and the concentration in the hepatopancreas showed a tendency to decrease with salinity. Results from gel filtration of the gill cytosol of the exposed carp indicated that the gills were more likely to be harmfully affected by exposure to an acute level of cadmium than the intestine tissue. In red sea bream, results from gel filtration of tissue cytosol suggested that cadmium accumulated in the intestine at a high level and the maximum detoxification capacity in this tissue was reached earlier than in the gill tissue. The metallothionein induced in the red sea bream liver by cadmium and zinc was separated into two isoforms by anion exchange column. However, the ratios of two isoforms when induced by zinc were different from those induced by cadmium.

INTRODUCTION

In recent years, people have been concerned that marine pollution by various pollutants is slowly advancing in extensive areas all over the world. In classical toxicology, a frequently used measure for the toxicity of a pollutant is the concentration which kills 50% of a group of animals within a given time period (LC50). In modem times, there is a need to develop assays for the degree of toxicity resulting from sublethal exposure to contaminants in the environment, since there may be no clear outward signs of toxicity in chronically exposed organisms.

Additionally, there is a need to develop contaminant-specific assays of toxicity since organisms may be exposed to various potentially harmful contaminants in the environment. Research on sublethal effects of contaminants has been attempted to date (Passino 1984) and various investigators have reported on their particular techniques for measuring the effect of pollution (Szumski and Barton 1983). Unfortunately, there has been little agreement among investigators about which techniques ate best for what and how they might be used for controlling marine pollution.

Heavy metals are among the most toxic and inevitable pollutants. There are several sources of heavy metal pollution, including mining industry, agricultural and silvicultural activities, waste disposal, and fossil fuel combustion. Cadmium is an extremely toxic element of continuing concern because its environmental levels have risen steadily with continued worldwide industrialization.

In order to understand better the impact of heavy metals, such as cadmium in aquatic organisms, it is important to understand the chemical and physiological processes that control their uptake, accumulation, storage and elimination. Although there have been many papers on cadmium accumulation and its toxicity to fish, most of them have dealt with freshwater fish. Perhaps it may be because the LC50 values of cadmium in saltwater fish are generally higher than those in freshwater fish. The marine environment may be deteriorating so that cadmium accumulation and its toxicity to marine fish must be further studied

The aim of this study is to clarify the characteristics of cadmium accumulation and its toxicity in marine fish.

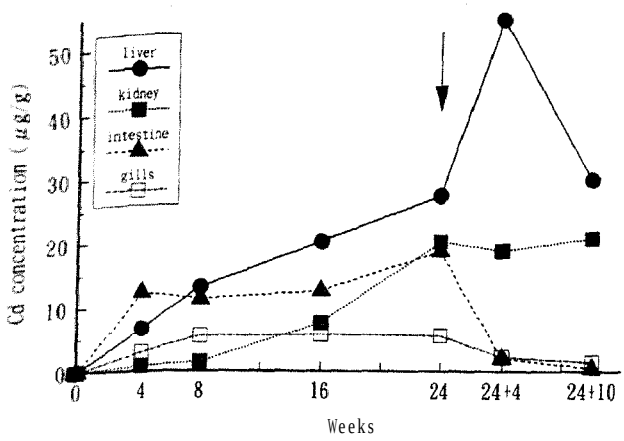

Fig. 1. Accumulation of cadmium in tissues of girella exposed to CD at 2.50 μg/L for 24 wk. The arrow indicates the end of exposure.

RESULTS AND DISCUSSION

DISTRIBUTION OF CADMIUM IN GIRELLA, *GIRELLA PUNCTATA*

Distribution of cadmium in the tissues of freshwater fish has been extensively studied (Nishihara et al. 1985). However, only limited data are available on cadmium accumulation in marine fish (Hilmy *et al.* 1985). Results from exposure of girella, *Girella punctata*, to Cd at 250 μg/L for up to 24 wk clearly showed that cadmium accumulates in the Livers and kidneys of marine fish (Kuroshima 1987) (Fig. 1). It should be noted that this accumulation was observed in the liver even after the end of exposure. In cadmium-treated rainbow trout, increase in total amount of cadmium in the liver and kidney after the end of exposure has been reported (Kumada et al. 1980). Cadmium content in the kidney and intestines of mice administered this metal by subcutaneous injections increased even after dosing ceased (Nicholson *et al.* 1984). Cadmium once taken up in the body thus appeared to be hardly excreted but redistributed among tissues. Cadmium detected in the intestines of exposed girellas is possibly due to the intake of water to maintain the water balance. Maximum cadmium accumulation in the gills of fish in Cd at 250 μg/L was less than twice that in Cd at 25 μg/L (data are not shown), indicating the tissue does not have capacity to retain the metal.

CADMIUM ACCUMULATION IN THE MUMMICHOG, FUNDULUS HETEROCLITUS, ADAPTED TO VARIOUS SALINITIES

Adult males and juveniles of mummichog adapted to freshwater and 5%, 10%, 15%, 20%, 25%, 50%, 75% and 100% seawater (v/v) were exposed to Cd at 1 mg/L for 24 h (Kuroshima 1992a). The hardness of freshwater and 25% seawater used in this study was approximately 45 mg/L and 250 mg/L as $CaCO_3$ respectively. Cadmium concen-

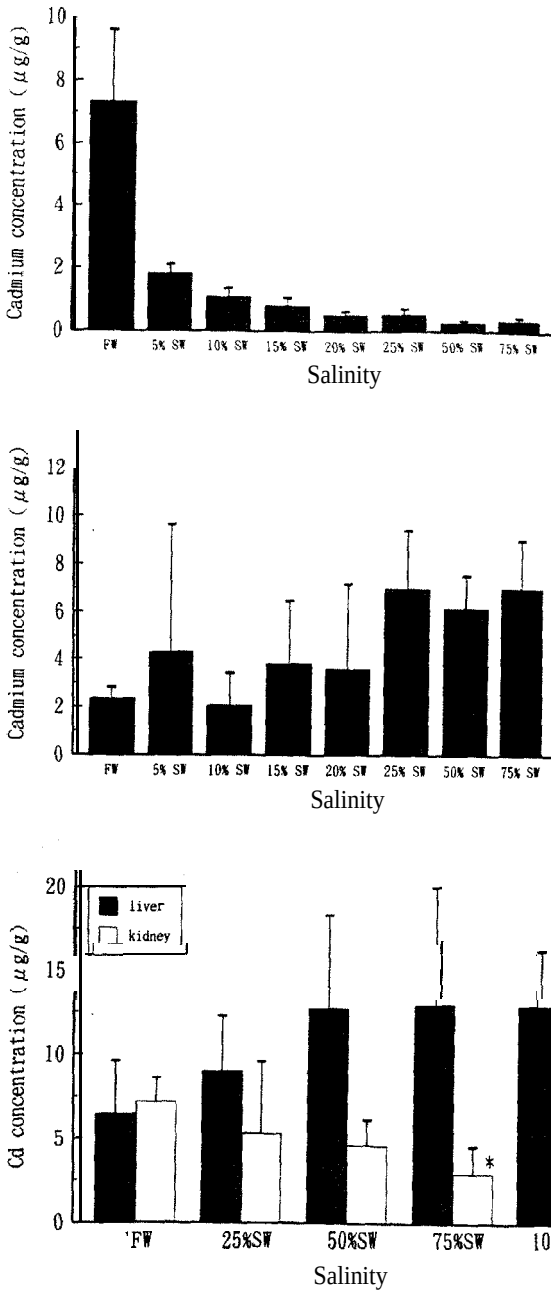

Fig. 2. Cadmium accumulation in tissues of mummichog adapted to various salinities: (a) the gills exposed to CD at 1 mg/L h; (b) the intestine exposed to CD at 1 mg/L for 24 h; and kidney and liver after intraperitoneal injection of Cd (0. BW) bound to metallothionein.

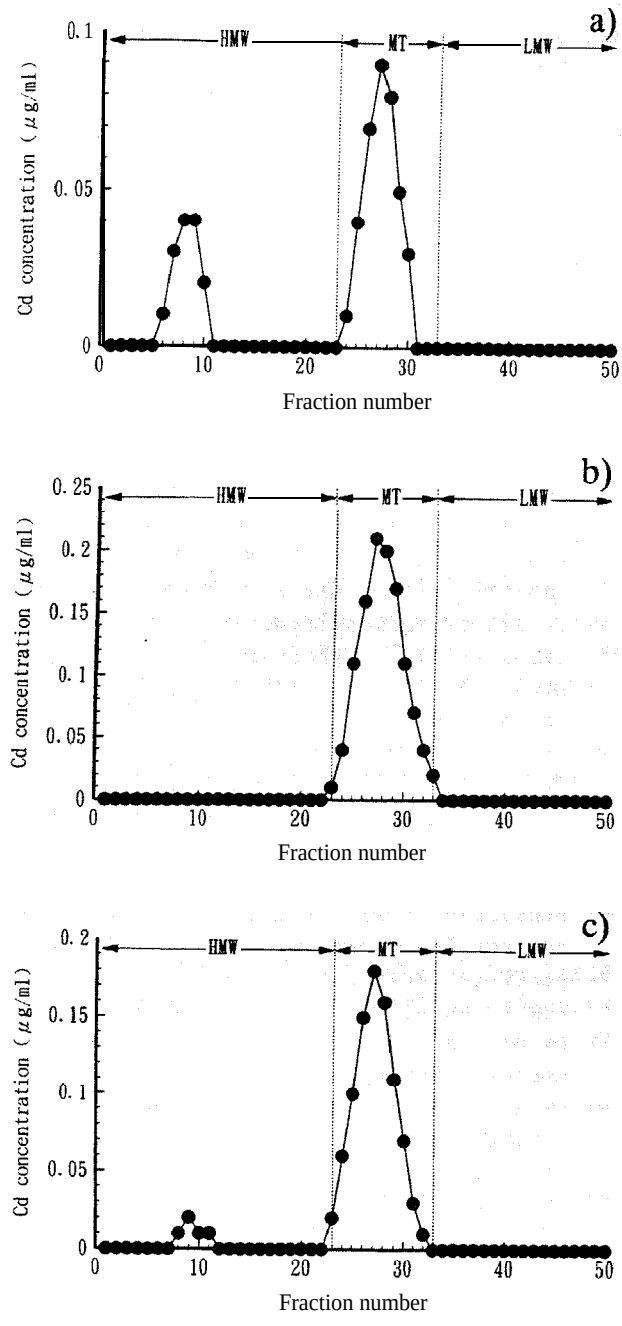

Fig. 3. *Geljiltration profiles of the tissue cystosolicfiaction: (a) the gills of carp exposed to Cd at 0.3 mg?l for 96 h; (b) the intestine of carp exposed to Cd at* 0.3 *mg/L for 96 h; (c) the gills of red sea bream exposed to Cd at 10 mg/L for 96 h; and (d) the intestine of red sea bream exposed to Cd at 10 mg/L for 96 h.*

trations in the whole body of juveniles, and in the hepatopancreas, kidney and gills of adult fish disproportionately decreased with increasing salinity, ranging from freshwater to 25% seawater and showed no further change at higher salinity (Fig. 2a). Depression of cadmium accumulation was most remarkable in the gills. Freshwater fish take up most of the ions necessary for homeostasis from water via the gills (Eddy 1982). Verbost at al. (1987) reported that Cd^{2+} readily enters branchial epithelial cells of freshwater fish, as does Cd^{2+} via La^{3+}- sensitive apical Ca^{2+} channels. Part and Svanberg (1985) proposed that gill permeability for cadmium decreases with increase in the hardness of water. The rapid decrease in cadmium accumulation in the gill, hepatopancreas and kidney of mummichog exposed in freshwater to 25% seawater was, thus, probably due to decrease in the active uptake of cadmium in the gills. The cadmium concentration in the intestine increased with salinity from freshwater to 100% seawater (Fig. 2b). Marine fish swallow seawater and absorb water together with monovalent ions from the intestine to compensate for water loss from the body. The higher concentration of cadmium in the intestine of mummichog adapted to the higher salinities may possibly have been due to the ingestion of water containing cadmium. Since water is actively, but cadmium poorly, absorbed from the intestine, cadmium dissolved in the water can be concentrated in the intestinal tract. In mammals, cadmium orally administered induces histopathological changes (Phillpotts 1986), inhibition of enzyme activity (Kobayashi and Kimura 1985), and transport of nutrients (Itturi and Pena 1986) in the small intestine. The intestinal function of seawater fish may possibly be damaged during exposure to cadmium.

Data from mammal experiments generally demonstrate cadmium bound to metallothionein to be less effectively trapped by the liver and mostly taken up by the kidney (Suzuki 1984). Results from the administration of cadmium bound to metallothionein by an intraperitoneal injection are shown in Fig. 2c. The cadmium concentration in the kidney decreased with increasing salinity and at salinities

of 75% and 100% seawater were significantly lower than those in freshwater. The cadmium concentration in the hepatopancreas showed a tendency to decrease with salinity. The ratio of cadmium concentration in the kidney to that in the hepatopancreas in each fish decreased with salinity. In the kidney of freshwater fish, essential electrolytes, glucose and vitamins are selectively reabsorbed from urine at the proximal tubules, while the main function of the kidney of marine fish is excretion of divalent cations. The function of the kidney to maintain the proper osmotic conditions in the body may, thus, possibly be related to the accumulation of cadmium bound to metallothionein in the kidney.

CADMIUM CYTOSOLIC PARTITIONING IN THE TISSUES OF CARP AND RED SEA BREAM

The carp, *Cyprinus carpio*, and red sea bream, *Pagrus major, were* exposed to Cd at 0.3 mg/L and 10 mg/L, respectively, for 96 h (Kuroshima 1992b). The cytosols of the gills and intestine were separated into fractions according to molecular weight using Sephadex G-75 gel chromatography. Cadmium in tissue cytosolic extracts were separated into a high molecular weight (HMW, >20,000 Da) pool, a medium molecular weight (MMW, 3,000-20,000 Da) pool and a low molecular weight (LMW, <3,000 Da) pool. The term Da denotes a unit for molecular weight of protein. The cadmium in the HMW pool containing enzymes and that in the MMW pool containing metallothionein was regarded as being potentially toxic and detoxified, respectively. Results from Sephadex G-75 gel filtration of the gill cytosol of the exposed carp indicate that the gills are more likely to be harmfully affected by exposure to an acute level of cadmium than the intestine tissue (Fig. 3a, b). In red sea bream, results from gel filtration of tissue cytosols indicate that cadmium accumulated in the intestine at a high level and the maximum detoxification capacity in the intestine was reached earlier than in the gills (Pig. 3c, d). It is reported that the intestine of scorpionfish may be the most highly influenced tissue on exposure to high cadmium concentrations (Brown et *al.* 1990).

METALLCYTHIONEIN INDUCED BY CADMIUM AND ZINC IN THE LIVER OF RED SEA BREAM

Metallothioneins are low molecular weight metalloproteins in the liver, kidney, and other tissues of various species including aquatic organisms (Unger et *al.* 199 1). They are characterized by unique amino acid compositions with a very high cystein content and the absence of aromatic amino acids (Fowler et *al.* 1987). Since metals like cadmium and zinc induce metallothioneins and bind to them via the sulfhydryl group of cysteine residues, they may possibly be involved in protection against cadmium toxicity and homeostasis of zinc and copper (Brady

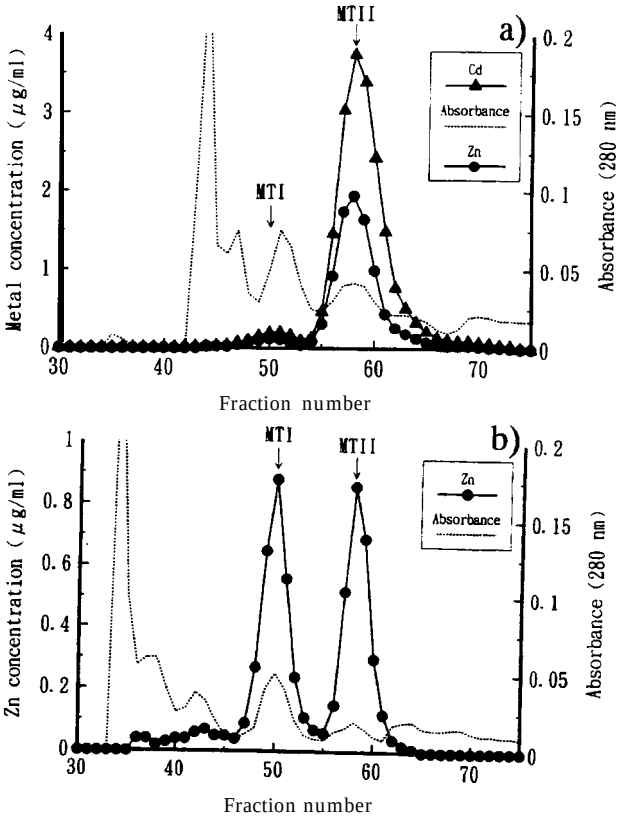

Fig. 4. Anion exchange chromatography of metallothionein-containing fractions from thegel filtration in the red sea bream liver: (a0 cadmium-induced metallothionein; and (b) zinc-induced metallothionein. The terms MIT and MTII denote isofonns of metallothionein.

1982). Recent studies have shown hormones (Hyllner et *al.* 1989). cytokines (Schroeder and Cousins 1990), and various forms of chemical and physiological stress (Kagi and Schaffer 1988) to induce metallothioneins and the expression of some genes to be under separate control and possibly to have different biological purposes (Sadhu and Gedamu 1988). The metallothionein induced in the liver of red sea bream by cadmium and zinc was separated into two isoforms by anion exchange column (Kuroshima 1995) (Pig. 4a). Following cadmium treatment, zinc was much higher in MTII than in MTI, where MTII and MTI denote isoforms of metallothionein. In metallothionein induced by zinc treatment, there were about equal amounts of zinc in both isoforms of metallothionein (Fig. 4b). These results suggest that the isoforms of metallothionein may have different biological functions.

CONCLUSION

Generally, when fish are exposed to pollutants, the organ or tissue most susceptible to adverse effects is the gills. This theory is based on results of experiments showing that gills are the main uptake site of many pollutants in

freshwater fish. However, the cadmium concentration in gills of girella exposed to the metal was low, and results from the examination of cadmium cytosolic partitioning in the gill tissue of red sea bream suggested that gills were not the most susceptible tissue. This discrepancy is considered to be partly due to the difference of osmoregulatory functions. The intestine is also an important organ for osmotic regulation of salt fish. The relative sensitivity of the intestine to cadmium exposure is of particular interest.

The liver and kidney are usually considered to be the principal sites of chronic cadmium toxicity. The ratio of cadmium concentration in the kidney to that in the hepatopancreas of the mummichog decreased with salinity tier an intraperitoneal injection of metallothionein. This result indicates that the ability to trap the cadmium bound to metallothionein in plasma by kidney is lower in saltwater fish than in carp. Therefore, the liver in saltwater fish may suffer chronic cadmium toxicity more severely than that in freshwater fish.

Metallothionein is thought to be involved in detoxification of certain heavy metals, such as cadmium, because of the avidity with which they bind to such metals. Iraddition, metallothionein may play an important role in the homeostatic control of zinc metabolism. Although it has been known that metallothionein is induced in fish, the information on this protein in saltwater fish is limited. Metallothionein in tissues of red sea bream was found to be present in two isoforms.

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Manipulation of Microbial Communities for Improving the Aquaculture Environment

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ABSTRACT

A microbial technique of biocontrol using the interaction of microorganisms to repress the growth of deleterious bacteria and viruses was developed. The bacterial strain used in this work also improved the growth of fishes and crustaceans. To control the bacterial population, protozoa was found having a significant role in the aquaculture biotope, The concept of biocontrol in aquaculture is also discussed in detail.

INTRODUCTION

Since there is a growing demand for animal protein, harvesting of biological resources by using advanced technology has extended to wider areas of the sea, resulting in the rapid depletion of marine resources. Mar-i- and aquaculture were developed in the 1960s to supplement and eventually replace wild catches. Today, the production of such fishes as yellow-tail and seabream, under rearing procedures, exceeds the amount captured in the sea in Japan. Furthermore, the harvest of the prawn, *Penaeus japonicus,* now extensively cultured in Japan, is equivalent to that in the sea. Cultured fish and prawn account for more than 10% of total yield and about 25% of total profit in the Japanese fisheries industry.

One of the major problems in the culture of fish is the development and rearing of larvae. Fish larvae often die in less than 24 h if adequate food is not available, since fry have small toothless mouths and are not mobile enough to catch food. For this reason, food size should be smaller than their mouth parts and be located near the fish. If fish larvae could be kept in enclosures away from their predators and receive an adequate supply of food, their survival rates would be much higher than those in the sea.

In this paper, several processes involved in the formation of microbial food assemblages in the food chain of the sea are described. According to this new concept of microbial food chain, fish production increased *in situ* in aquaculture using bacteria and protozoa as live feeds.

DISTRIBUTION PATTERN OF BACTERIA IN AQUACULTURE

In the natural sea if the concentration of bacteria reaches more than 10^6 cells/ml, protozoa grow quickly and eat bacteria to reduce the number to around 10^6 cells/ml (Maeda and Liao, 1994). In aquaculture, the same ecological processes are present and approximately the same biomass of bacteria, 10^6 cells/ml, can be measured, For example, in the culture of the crab, *Portunus trituberculatus*, the bacterial strain, PM-4 (*Thalassobacter utilis), was* added to repress the growth of pathogenic microorganisms and to promote fish growth (Nogami and Maeda, 1992). At first, the crab at its Zoea I stage fed on bacteria which resulted in a decrease of bacteria just after the addition of *T. utilis.* Since larvae started eating other feed after Zoea I, the bacterial number increased to more than 10^7 cells/ml. At this period, protozoa increased quickly and fed on bacteria to reduce its number to around 10^6 cells/ml (Nogami and Maeda, 1992).

BACTERIAL FLORA

Among aerobic bacterial assemblages, species belonging to the genera *Pseudomonas, Vibrio, Acinetobacter, Alteromonas* and *Flavobacterium are* common in aquaculture water. *Vibrio* spp. can grow especially quickly because of their high growth rate and because of their ability to adapt to an oxygen deficient condition. Consequently, the main pathogenic bacteria infecting fish are the *Vibrio* group.

The rotifer, *Brachionus plicatilis,* which is used as a live feed for fish, is cultured with the microalgae, *Nannochloropsis oculata.* Certain species of bacteria very often cohabit with these algae. For example, the pigmented bacteria, mainly the species *Flavobacterium,* attach to this microalgae. When rotifers are cultured with N. *oculata*, *Flavobacterium* spp. attached to this microalga tend to inhibit the growth of the rotifers when they eat *N. oculata.*

The water in which rotifers are cultured contains a high concentration of dissolved organic matter derived from the food and feces of rotifers. This results in a decrease in the oxygen content as a result of bacteria and other microorganisms inhabiting together. As mentioned above, in this sort of environment *Vibrio* spp. can grow faster than other

bacteria and eventually dominate the bacterial communities. Also, for the same reason, *Vibrio* spp. are abundant inside the gut of rotifers. Among these *Vibrio* populations, pathogenic species may occur and infect the fish and crustacean larvae. Thus, specific bacterial species cohabiting with microalgae and rotifers are not always useful to the organisms which feed on these algae and rotifers.

EFFECT OF STERILIZATION OF SEAWATER ON MICROORGANISMS

The method for keeping pathogenic microorganisms away from fish rearing environments is one of the main concerns of people engaged in aquaculture. For this purpose, the filtration of water, the addition of sodium chloride, ozonation, the use of ultraviolet tight and even the use of artificial compound food containing antibiotics for sterilization, are all commonly adopted techniques in aquaculture. People in aquaculture tend to believe that these Procedures can eventually eliminate all microbes in seawater and produce and maintain a nearly pure water quality. However, with these treatments the occurrence of Pathogenic microorganisms which causes fish diseases cannot be permanently removed from the water. For example, if the antibiotic kanamycine (50 mg/L) is added to seawater, bacterial numbers decrease for about two days, but eventually the numbers will recover to the original level, 10^6 cell/ml. The same phenomena can be seen when seawater is sterilized with filtration, ozonation or ultraviolet light treatment. After such treatment, bacteria grow very quickly because there is less antagonism among the bacterial populations present. Furthermore, no one can anticipate what kind of bacterial species may grow in the vacant space produced by the above treatments. For example, in Japanese aquaculture hatcheries, when the crabs, *Portunus trituberculatus*, were infected by pathogenic *Vibrio* sp., various kinds of antibiotics were added to the larvae rearing water. At first, the reagents repressed the growth of pathogens, but after about 10 h drug resistant microbes, mainly fungi, appeared and grew quickly, killing all the larvae. Because this same sequence of events was experienced repeatedly, when infected larvae are found now, larval Production is stopped, all the larvae are thrown away and the Procedure begins again.

Although people who engage in aquaculture have started to realize that antibiotics are less effective, almost no alternative method of controlling diseases has been found. It is therefore essential that new methods be adopted wherein the antagonism of certain microorganisms is used to repress other pathogenic microbes in seawater.

The antagonism among microbes is a naturally occurring phenomenon through which Pathogens can be killed or reduced in number in the aquaculture environment. This method is called biological control, or biocontrol. The term biocontrol is becoming familiar in agricultural science. Commercial success has been obtained in controlling the ubiquitous crown gall disease by using an avirulent strain of *Agrobacterium tumefaciens* (also referred to as *A. radiobacter*) (Kerr, 1972, 1980). The health of organisms in nature depends primarily upon their inherent resistance to microbial invasion and the biological equilibrium between competing beneficial and deleterious microorganisms at the interface of the organism as mediated by the environment. In aquaculture, some microbes play a major role in this equilibrium in their interactions with various pathogenic agents.

SITES FOR ISOLATING THE USEFUL BACTERIA FOR BIOCONTROL METHOD

Among the many species of bacteria distributed in seawater, only some species are useful for fish production. For example, many bacteria inhibit the growth of fish larvae. When bacteria are isolated using the agar plate technique, there might be more than 10 kinds of bacteria on the plate. In some aquaculture environments, several hundred strains can be easily collected using many agarplates. To determine the activities of these isolated strains on fish growth or on inhibition of pathogenic microorganisms, it takes a considerably long period. Therefore the sampling site should be selected to isolate useful bacteria with high efficiency. Possible sites are as follows: (1) seawater in *situ* where larvae are rearing; (2) seawater where larvae are being condensely reared in the laboratory; and (3) the digestive gut of fish.

Bacteria which promote the growth of fish are most likely to be found in an environment which fish inhabit. For this reason, fish rearing seawater is a good site for isolating useful microbes. In addition, seawater where high concentrations of larvae are being reared is an ideal environment for finding these bacteria However, one should be careful not to isolate harmful bacteria if it becomes apparent that the conditions of the larvae are deteriorating. in the rearing container. It is also possible to isolate bacteria inside the gut of fish, but based on our research useful bacteria are seldom found this way.

CONSTRUCTION OF PRAWN CULTURE SYSTEM *IN VITRO* AND ISOLATION OF USEFUL BACTERIA

The useful bacteria to promote the growth of prawns tend to cohabit with prawns in seawater. The construction Procedure to rear prawn larvae in the laboratory is mentioned next.

A 100 L bottle is prepared under a light source of about 3,000 lux at 25°C. Five L of seawater sterilized with filtration by Millipore HA type filter (pore size: 0.45 μm) are stored inside the bottle. Prawn larvae which should be obtained from the hatchery where only naturally matured spawners are used to produce eggs are added to seawater

Table 1. Survival and moulting rates of the larvae, *P. Monodon*, with and without soil extract

Experiment	Survival rate (%)						Moulting rate to ZIII (%)		
	Oday	2nd	3rd	4th	6th	7th	0 day	6th	7th
I	100	100	100	*76*	*61*	*61*	*0*	51	85
II	100	*56*	*53*	*52*	*50*	*50*	*0*	*0*	*0*
III	100	50	0	0	—	—	0	—	—

Experiment I: with diatom and soil exract
Experiment II: with diatom and without soil extract
Experiment III: without diatom and soil extract
(ZIII: Zoea III stage)

at a concentration of 100 ind./L. All larvae in this study should be the firstborn from a spawner. A diatom, *Navicula* sp., about 10μm in length and grown in liquid medium at a concentration of 10^4 cell/ml is added to larvae rearing water. Instead of *Navicula* sp., *Chaetoceros gracilis can also* be used. Finally, a soil extract at a concentration of 5% (v/v) is added. To prepare the soil extract, soil under a lawn free from herbicides at least for one month is mixed with twice the volume of distilled water and autoclaved for 30 min at 121°C. After cooling, the soil suspension is filtered through a paper filter and the filtrate is stored in the freezer at -20°C. A small amount of Bacto-peptone (O.Ol%, Difco Co. Ltd.) can be used instead of soil extract.

Three experiments are going to be conducted, where Experiment I contains soil extract and diatom, Experiment II contains diatom only, and Experiment III contains neither soil extract nor diatom. The activity of prawns is determined in terms of moving ability to the light source which is set at one side of the transparent container where larvae were kept (Maeda and Liao. 1992).

The example data of Maeda and Liao (1992) are shown as follows (Table 1). The survival rate of the prawn larvae is a little higher in Experiment I than in Experiment II, while prawn larvae in Experiment III all died within three days. Although survival rates in both Experiment I and II showed little difference, the molting rate of a prawn of the growth stage from Protozoea II to III was much higher than in Experiment II, in which 85% of the larvae molted to Protozoea III stage within seven days. Since food was supplied only at the beginning of this experiment, the growth rate was not as fast as usual. However, it would seem that the addition of a soil extract, as a source of organic matter for sustaining the growth of microorganisms, stimulates the molting of prawns. One of the agents which stimulates the growth of prawns is microorganisms multiplied utilizing mainly soil extract in the experiment.

From seawater in Experiment I, seven strains of bacteria were isolated. The isolated and purified strain was added to the prawn culture water of *Penaeus monodon* (100 ind./L) at a concentration of 108 cells/ml with a diatom, *Navicula* sp. (10^4 cells/ml). The survival and molting rates of larvae were determined. Most bacterial strains tested were not effective in promoting the growth of the larvae, but strain PM-4 gave higher survival and molting rates for the larvae compared to those in the control experiment which contained only a diatom. The activity of the larvae was also higher in the presence of the PM-4 strain (Maeda and Liao, 1992). All the experiments carried out used the crab larva *Portunus trituberculatus*, but similar results were obtained in experiments using *P. monodon*.

REPRESSION OF THE GROWTH OF PATHOGENIC MICROORGANISMS

Two rectangular smears of bacteria to be tested (4 cm in length with a 3 cm gap between the smears} were made on a plate of 2216E Marine Agar (Difco Co. Ltd), and a 2 cm-long rectangular smear of *Vibrio anguillarum*, a pathogenic bacterium of fish, was made between the two larger smears. To determine *Vibrio* static activity, the width of the smear of *V. anguillarum was* measured. The bacterium in this test system was incubated for certain periods. In this assay, the bacterium PM-4 repressed the growth of this pathogen (Maeda, 1994).

PROCEDURES FOR REARING LARVAE USING BACTERIA

The useful bacteria which are cultured in a large scale bottle are going to be used *in situ* in a larvae rearing environment. The detailed procedures to rear the larvae of the crab, *Portunus trituberculatus*, are described here.

Spawning females are kept in a container of two metric tons, with a water depth of 30 cm, in sand and circulating 25°C seawater (filtered with sands of about 400 μm in diameter). Mature females were transferred from this tank to a 700 L gentle aerated container about 24 h before the larvae were hatched out.

Seawater used for crab culture (150 m^3) is filtered with sand (grains average 400 μm in diameter) and sterilized with sodium hypochlorite (50 mg/L) before adding the

bacterium. This procedure, which eliminates almost all naturally occurring bacteria, is followed by neutralization with sodium thiosulpbate at the beginning of the experiment.

To 200 m^3 of seawater used for rearing the crab larvae, 15 L of the bacterial culture solution were added once a day for 7 days, which resulted in initial bacterial concentrations of about 10^6 cells/ml. Crab larvae (28,000 ind./m^3), diatoms (1,200 cells/ml) and rotifers (5,000 ind./L) were added to culture water on the first day of the experiment.

In the experiments with the biocontrol method, all the larvae survived. On the other hand, without the PM-4 strain, almost all the larvae died on reaching Megalopa I (Nogami and Maeda, 1992).

BIOCONTROL OF VIRAL DISEASES

Several viral diseases have seriously affected the fish rearing industry. Viruses are the smallest infectious agents (20-300 nm in diameter), usually as a single molecule containing DNA or RNA. Vises replicate only in living cells. During the replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat proteins assemble together to form the capsid, which encases and stabilizes the viral nucleic acid against the extracellular environment and facilitates the attachment and perhaps penetration of the virus upon contact with new susceptible cells. Viruses are known to infect unicellular organisms such as mycoprasmas, bacteria, algae, and all higher plants and animals. As a basis for the classification of viruses, nucleic acid type (RNA or DNA, single-stranded or double stranded) size and morphology, presence of specific enzymes, add immunologic properties can be listed.

The concentration of viruses in natural waters is generally believed to be low (Hidaka, 1977), and they have therefore been considered ecologically unimportant. Bergh et *al.* (1989), using the direct counting method of the electron microscope, reported virus counts ranging from 10^6-10^9 virus particles/ml. These counts were Id-10^7 times higher than previous reports on virus numbers in natural aquatic environments, which in turn were based on counts of plaque-forming units using various host bacteria (Hidaka, 1977). They also found that most of the virus particles appeared to be free in the water, but some were associated with bacteria. Suttle et al. (1990) and Nagasaki *et al.* (1993) found virus particles inside the cell of microalgae. They suggested these viruses might greatly affect the mortality of the microorganisms present together.

In considering methods for keeping pathogenic viruses away from fish rearing environments, the above mentioned studies are very important. In the results, the concentration of viruses changed from 10^3 to 10^9 virus particles/ml, and many of the viruses were present as a free form in seawater. These results suggest the presence of antivirus microorganisms in seawater. It also helps to explain why the concentration of viruses fluctuated so greatly. In addition, viruses will transfer from one infected organism to another through the water environment. If antivirus bacteria dominate the water environment, virus infection among fishes may be repressed to a large extent. Based on this concept, antivirus bacteria are used in the larvae rearing procedures ***in situ*** aquaculture as mentioned next.

DETERMINATION OF ANTIVIRUS ACTIVITY

Usually bacteria which have activity to repress the growth of other bacteria can inhibit the virus growth. Therefore when an antiviral bacterium is going to be found in seawater, anti-bacterial activity can be determined. This procedure is easier than determining the antiviral activity directly. Therefore checking for antiviral activity may not always be necessary for those who want to avoid the following complicated procedures.

To cultivate a pathogenic virus, a living fish cell is required, usually an epidermal carp cell (EPC), an epidermal king salmon cell (CHSE2 14) or cells from the gill of the bluegill (BF2) are commonly used. The combination of an EPC cell and Infectious Hematopoietic Necrosis Virus (IHNV) is considerably easier to be used in the antivirus assay procedures. An EPC cell is grown in MEM-IO medium, which is composed of 10% fetal bovine serum (M.A. Bioproduct), 0.075% $NaHCO_3$, 100 IU/ml penicillin (Sigma), 100 µg/ml streptomycin (Sigma) and 1.6% Tris-buffer (Tris(hydroxymethyl)amino methane(Tris)-hydrochloride) (Sigma), pH7, 8 at 15°C. The cells are seeded in 1 ml of the growth medium per well, in a 24-well(16 mm in diameter. Falcon) plate, to give approximately 10^6 cells/ml/well. The IHN virus is first propagated in rainbow trout gonad cell, RTG-2, in a 75 cm^3 2-plastic flask (Falcon) containing 25 ml of MEM10-Tris; this virus is inoculated in the well where EPC is grown. After a cytopathic effect develops and the cells desquamate, the cells are centrifuged at 4,000 rpm at 4°C for 30 min. The supernatants are filtered with a 0.45 µm pore size Millipore filter (HA type). The supernatant containing virus is stored at -80°C until use.

Bacteria tested are. grown in a 100 ml ZoBell 2216E medium for 3 days. The culture is centrifuged and the supernatant is filtered with a 0.22 µm pore size Millipore filter. Equal volumes of bacterial filtrate and IHNV virus suspension are mixed for 3 h at 20°C. The reaction mixture, 0.1 ml, is inoculated into the well in which the EPC cells with 1 ml of MEM- 10 are already placed. Virus suspension used should be diluted for certain concentrations and submitted to this test. The reference test which contams no virus suspension is set at the same time. In each well, cytopathic effect (CPE) is determined under the microscope.

The Strain VKM-124 which has the *Vibrio* static activ-

ity and is used *in situ* in aquaculture, clearly repressed the appearance of CPE on the EPC cell in which a maximum 3 days delay was observed in the VKM- 124 experimental fraction compared in the reference fraction. Thus, the strain which has the *Vibrio* static activity represses the growth of virus at the same time.

There are many viral diseases. *Penaeus* prawn, *P. monodon* and *P. japonicus* have almost all been infected by Baculo-like viruses. There are also other viruses: Infectious Hematopoietic Necrosis Virus (IHNV) and Infectious Pancreatic Necrosis Virus (IPNV) which infect salmon; Viral Haemorrhagic Septicemia Virus (VHSV) which infects trout; the Yellowtail Ascites Virus (YAV) which infects yellowtail; Sima-aji Neuro Necrosis Virus (SJNNV) which infects Guelly Jack; the Iridovirus which infects seabream; and many more, all of which cause serious damage in aquaculture. The bacterial strain VKM- 124 used in the aquaculture processes shows the effective activity to repress the SJNNV, Baculo-like viruses. This strain is being used in seawater at the concentration of about 10^6 cells/ml in larvae rearing containers of Sima-aji (Fig. 1).

If grown fish, which feed on artificial compound food (AFC), are to be protected from viruses, the bacterial culture liquid is mixed with the AFC at the ratio of 20% (v/v) of AFC.

CONCLUSION

In aquaculture water if the concentration of bacteria reaches more than 10^6 cells/ml, protozoa grow quickly and eat bacteria to reduce the number to around 10^6 cells/ml.

When seawater is sterilized with reagents (antibiotics et *al.),* filtration, ozonation or ultraviolet light treatment, after such treatment bacteria grow very quickly because there is less antagonism among the bacterial populations present. Furthermore, no one can anticipate whether pathogenic bacterial species may grow in the vacant space produced by the above treatments.

People who engage in aquaculture have started to realize that antibiotics are less effective; almost no alternative method of controlling diseases has been found. It is therefore essential that new methods be adopted wherein the antagonism of certain microorganisms is used to repress other pathogenic microbes in seawater.

From seawater many bacterial strains were isolated. The isolated and purified strain was added to the prawn culture water of *Penaeus monodon (100* ind./L) at a concentration of 10^8 cells/ml with a diatom, *Navicula* sp. (10^4 cells/ml). The survival and molting rates of larvae were determined. Most bacterial strains tested were not effective in promoting the growth of the larvae, but strain PM-4 gave higher survival and molting rates for the larvae compared to those in the control experiment which contained only a diatom. The activity of the larvae was also higher in the presence of the PM-4 strain.

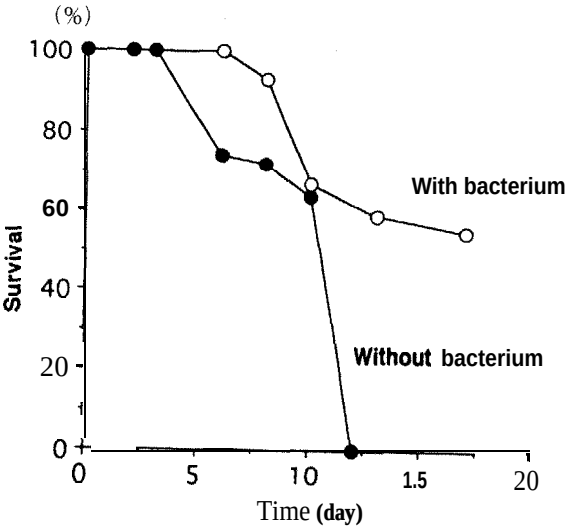

Fig. 1. Biocontml of virus diseases in yellowjack (shima-aji) culture water: In 20 *metric tons of culture water: 250,000 larvae* were *kept and the bacterial strain was added at the concentration of about 10^6 cells/ml.*

The bacterium PM-4 also repressed the growth of the pathogenic bacterium *Vibrio anguillarum.*

In the experiments of rearing the crab, *Portunus trituberculatus,* with the biocontrol method using the bacterial strain PM-4, all the larvae survived. On the other hand without the PM-4 strain, almost all the larvae died on reaching Megalopa I.

The strain VKM- 124, which has the *Vibrio* static activity and is used *in situ* in aquaculture, clearly repressed the activity of Sima-aji Neuro Necrosis Virus (SJNNV).

When the bacterial strain VKM-124 is being used in seawater at the concentration of about 10^6 cells/ml in larvae rearing containers of Sima-aji, 60% of the larvae survived. On the other hand, without the strain all the larvae died within a short period.

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Resolution of Sustainability Issues in South Carolina Shrimp Aquaculture: Progress to Date and Future Direction

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ABSTRACT

Uncontrolled development of shrimp aquaculture industries has the potential to adversely impact sustainability of natural resources such as land, water and aquatic biota, energy, and sources of protein. The small shrimp farming industry in South Carolina does not tax available resources but, to ensure that the industry is able to continue to grow, efforts are underway at the Waddell Mariculture Center to develop technology which makes more efficient use of these resources. Land use was effectively addressed through development of intensive systems with demonstrated production capabilities of 34 metric tons/ha/crop, but it was found that super-intensive systems may exacerbate water use problems, with the primary concern being the discharge of pond water to adjacent coastal waters. Studies found that elimination of routine water exchange does not significantly affect growth or survival as long as dissolved oxygen concentrations are maintained and the feed inputs do not exceed the assimilative capacity of the pond. To date, production levels of 7-8 metric tons/ha/crop have been achieved without water exchange and the water has been reused for a subsequent crop. Planned research and development activities are expected to increase the production capacity of no-exchange systems to at least 10 metric tons/ha/crop. There are indications that elimination of water exchange and continuous water reuse may facilitate nutrient recycling and increase the conversion efficiency of shrimp feed protein to shrimp biomass protein. Modified feed management regimes, low-protein feed formulations and the use of secondary crops of herbivorous fish and mollusks to convert waste nutrients to edible seafood products are also being used to improve protein transformation efficiencies. Future research and development activities are expected to show that protein transformation efficiencies for integrated multi-species systems with complete water reuse can be 30% higher than traditional intensive shrimp farming technology. The energy requirements of these systems are being modeled. The aeration energy requirement is a function of the feed input, so improvement in feeding efficiency also improves energy efficiency. The aeration energy requirements may increase slightly when water exchange is eliminated, but the total energy inputs (aeration vs. aeration and pumping) are the same. Planned research and development work will incorporate continuous monitoring and automated equipment switching to further improve energy efficiency.

INTRODUCTION

South Carolina, on the Atlantic Coast of the United States, has a small, modestly profitable shrimp farming industry. Fifteen farms, most of which are owner-operated, produce about 450 metric tons annually in about 100 ha of ponds. One crop is produced in the 4-6 month growing season. Since the stocking window is very limited, and South Carolina has little hatchery production, most of the postlarvae are imported from hatcheries in other parts of the United States.

Production collapses of poorly regulated shrimp aquaculture industries in Taiwan (Chen 1995), Thailand (Lin 1995), Ecuador (Stem 1995), Indonesia (Winarno 1995) and China (Wang et *al.* 1995) provide stark examples of what can occur when unsustainable, highly resource-dependent production systems are allowed to proliferate at will. The impacts are felt by both the shrimp farmer and other users of public water resources. Systematic regulation and permitting with a sound scientific basis is needed to protect the environment and its dependent industries. While its shrimp farming industry is small with negligible environmental consequences, South Carolina has become a leader in creation and adoption of sustainable production practices. This situation has developed in response to the high value placed on the nearly-pristine nature of the coastal environment, and the fact that the state's Depart-

ment of Natural Resources is the lead agency in aquaculture research and development, and operates the Waddell Mariculture Center (WMC).

Four factors affect aquaculture's natural resource sustainability: (1) land use; (2) water use; (3) energy use; and (4) protein transformations (Hopkins, in press). Unfortunately, optimizing the sustainability of one of these four factors may adversely affect another as there are significant interactions between them. For example, intensive culture practices puts available land to its best use, but may exacerbate problems with water use, energy use and protein transformations. To varying degrees, research and development programs at the WMC in South Carolina are addressing all of these resource sustainability factors. A holistic approach is being used, with the ultimate objective being creation of integrated systems with the least overall reliance an natural resources (Sandifer and Hopkins, in press). This paper discusses the progress to date and summarizes future direction and related research planned for the next several yr.

PROGRESS TO DATE

LAND USE

Coastal land in South Carolina has traditionally been used for agriculture and silviculture, although the Charleston area has a long history of various tight and heavy industries. Most of the present-day development along the coast is associated with residential and retirement communities, tourism and services which support these activities. As a result, the amount of coastal land under cultivation is declining. Demands for land for community and tourism development have caused land prices to escalate and increased the tax burden. Thus, rural land owners must either search for more lucrative agricultural opportunities or sell their land. The longer they meet their tax obligations and stave off development, the more valuable their land becomes.

Land parcels in coastal South Carolina are generally small, partially as a legacy of land reforms which occurred 130 yr ago near the end of the U.S. Civil War. The intensive shrimp production practices used by most farms is a reflection of the generally small size of individual parcels, coupled with high land costs. The land resources needed for extensive culture do not exist, except for some difficult-to-manage intertidal wetlands which were impounded over a century ago for rice farming and were, until recently, maintained for waterfowl management. Thus, shrimp farming in South Carolina tends to be either very intensive, or very extensive, with little intermediate activity. With research and development support from WMC, shrimp farms are able to achieve yields considerably higher than those found anywhere else in the western world (Hopkins 1991).

The pioneering work in development of intensive culture practices for western white shrimp species is that of Sandifer *et al.* (1987, 1988, 1991a, b). Sandifer *et al.* (1987) demonstrated that white shrimp can be intensively cultured in ponds provided with supplemental aeration and water exchange. Sandifer et al. (1988) found that density has a minimal effect on growth and survival, indicating that hyperintensive systems may be viable if water quality is maintained. Indeed, Sandifer *et* al. (1991a, b) later demonstrated production as high as 2 1.3 metric tons/ha. Since that time, further pressing of the production capacity of intensive ponds has yielded 34.2 metric tons/ha (WMC, unpublished data). While the average production of commercial ponds (other than impoundment ponds) is 4-5 metric tons/ha/crop, some South Carolina commercial farms have operated at production levels of up to 13 metric tons/ha (Sandifer et *al.* 1993). Without this intensive technology, shrimp farming in South Carolina would probably not be economically viable today. However, while intensive culture practices have allowed the industry to form in a way that makes efficient and sustainable use of land resources, present pond management practices are less than satisfactory. Pond management practices have not made the best use of water resources, energy resources or protein transformations.

WATER USE

Initially, water use in intensive pond production was fairly high. Phillips et *al.* (1991) noted that production of a metric ton of shrimp uses 16,000, 36,000 and 55,000 metric tons of water for extensive, semi-intensive and intensive systems, respectively. These were the highest water use values reported for a variety of aquaculture crops considered. Hopkins and Villalón (1992) surveyed water usage in commercial shrimp farming worldwide and found no correlation between water exchange and production goals. Sandifer et *al.* (1988, 1991b) reported average water exchange rates of 8-23% of the pond volume a day while Sandifer et al. (1991a) reported 8-16% a day and noted that similar intensive production trials in Hawaii used 61% a day water exchange. In general, shrimp farmers are using water exchange rates which are known to produce acceptable results, without systematically determining the minimum requirement.

Thus, Hopkins *et al.* (1991a) compared the effect of "normal" (14%/day) and "low" (4%/day) water exchange on growth and survival of *Penaeus vannamei* stocked at 76/m^2 and found no differences. Browdy **et al.** (1993) examined the effects of water exchange rates of 10, 50 and 100% a day upon growth and survival of *P. vannamei* stocked in outdoor tanks at 60 and 100 shrimp/m^2 and concluded that water exchange rates of 10 to 100% a day have little impact upon growth or survival as long as dawn dis-

solved oxygen levels are maintained at acceptable levels. Hopkins et al. (1993a) determined the effects of high (25%/day) and reduced (2.5%/day) water exchange on water quality and production in intensive shrimp ponds stocked with *P. setiferus* at 44 postlarvae/m^2 and found that, even though nutrient concentrations were higher in ponds with reduced water exchange, the total mass of pollutants discharged to the environment was reduced. Mass balance calculations for nitrogen in the system indicated that the amount of nitrogen added to the system as feed and subsequently lost through nitrification and atmospheric diffusion was 16% and 43% a day for the high-exchange and reduced-exchange ponds, respectively.

Since the pond ecosystem appears to be a fairly efficient mechanism for assimilation of waste nutrients, production trials assessing the feasibility of completely eliminating water exchange were begun. Hopkins et al. (1993a) compared water quality and shrimp production in ponds stocked with *P. setiferus* postlarvae at three densities (22, 44 and 66/m^2) and operated with no water exchange. Feeding rates were proportional to the stocking density but varied dramatically over the production period. Production was excellent in the low density pond. However, ponds stocked at 44 and 66/m^2 experienced mass mortalities. Mortalities could not be attributed to a toxic effect of any one water quality parameter, and gill fouling or other disease agents were suspected of being the cause of unusual mortality. Hopkins et *al.* (unpublished) compared ponds stocked with *P. vannamei* postlarvae at 38/m^2 with and without water exchange. There were three replicates of each treatment and ponds with water exchange received 15% of the pond volume per day beginning on day 42. In this study, survival was excellent in all ponds. Average growth was slightly higher in the ponds without water exchange but there was much overlap and differences were not significant. Shrimp production without water exchange was 5-6 metric tons/ha. Hopkins et al. (1995a) investigated the effect of low-rate coarse-grain sand filtration on water quality and production in static and recirculating no-exchange ponds. The static control pond demonstrated excellent survival and normal growth with production levels which approached 7 metric tons/ha/crop without water exchange. Compared to the initial studies with decreased or eliminated water exchange (Hopkins et *al.* 1993a), the application of feed in these subsequent studies was stable with the same amount of feed applied each day. Since that time, Hopkins et *al.* (1995b) demonstrated production levels as high as 8.2 metric tons/ha/crop without water exchange. Water from these trials was saved during the harvest process and used to grow a second crop in the following year, still without deleterious effects caused by water quality deterioration (Hopkins et *al.* 1995c). While the maximum production level of shrimp ponds operating without water exchange has not yet been determined, there is some evidence that the ceiling may be as high as 9-10 metric tons/ha/crop.

Elimination of water exchange resolves numerous problems associated with the environmental impacts and sustainability of shrimp farming. The most often-cited problem is the eutrophicating effect of pond discharges on the adjacent estuarine ecosystem (Schwartz and Boyd 1992). However, other concerns, such as entrainment of marine organisms by seawater pumps and escapement of aquaculture stocks into the marine environment, are also effectively addressed when water exchange is eliminated (Hopkins et al. 1995d). Both issues, entrainment and escapement, have been raised by regulatory agencies, commercial fishing interests and the public at large in South Carolina.

The implications of the release of the non-native *Penaeus vannamei* into habitats of the indigenous *P. setiferus*, *P. aztecus* and *P. duroarum*, especially if the aquaculture stock may be carrying diseases which are not found in local wild stocks, have actually been the greatest concern associated with the shrimp farming industry in South Carolina (Wenner and Knott 1992). This problem has been addressed through regulation on the state level. The state's Department of Natural Resources requires a permit for the importation of postlarvae, and the permit is issued only after the hatchery demonstrates that the postlarvae they produce are free of pathogens which could impact aquaculture and wild stocks. In addition, new screening regulations have been imposed and are strictly enforced to minimize or eliminate the degree of escapement from aquaculture facilities.

ENERGY USE

Energy requirements for shrimp farming increase as the production goal and feeding rate increase. When feed rates exceed 14-18 kg/ha/day, biochemical oxygen demand may exceed the pond's reaeration capacity. When the total oxygen demand exceeds diffusion and photosynthetic oxygen surpluses, dawn dissolved oxygen concentrations drop to levels which are lethal to shrimp. This oxygen deficit must be counteracted by either (1) exchanging water and transferring some of the oxygen demand to the adjacent estuary or by (2) using supplemental aeration. Both of these measures are energy-intensive; however, water exchange has the obvious disadvantage of both increasing energy use and abusing the adjacent estuarine ecosystem.

Until recently, all farms used water exchange and most use supplemental aeration when production goals exceed about 2 metric tons/ha. An empirical model of the aeration requirement for a given feeding rate and minimum acceptable dawn dissolved oxygen concentration for ponds with an average of about 15% a day water exchange was prepared (Hopkins *et* al. 1991b). Since managing ponds without water exchange is a fairly new practice, we do not

have a data set large enough to prepare a valid empirical model for aeration requirements of these systems. However, preliminary indications are that the aeration demand will not increase more than a few percent when water exchange is eliminated in intensive systems. For most pumping systems, energy requirement for water exchange is higher than the energy required to increase aeration by at least 10%.

There is, however, room for additional cuts in energy use in intensive shrimp farming. While generalized nighttime aeration requirements are fairly well known, the dynamic nature of pond ecosystems changes the aeration requirements from day-to-day and h-to-h. There is variability among farms in the number of hours aeration equipment is run each day. This variability in the hr of operation is influenced by (1) the cost and limited availability of dependable, real-time oxygen monitoring and automation equipment; (2) a general lack of knowledge about the relative importance of water column mixing (aeration equipment both mixes and aerates); and (3) a general lack of understanding of the importance of volatilization of certain nutrients and diffusion gases other than oxygen.

Labor costs constrain continuous manual oxygen monitoring using typical handheld polarographic meters. Continuous oxygen monitoring equipment is plagued by fouling of semipermeable membranes in the microbial-rich pond water. The technology for dependable self-cleaning oxygen probes is improving, but the required investment for equipment is currently beyond the means of most shrimp farms. Once inexpensive and dependable continuous oxygen monitoring equipment is available, aeration equipment can be automatically activated or deactivated in response to instantaneous oxygen needs.

Without horizontal and vertical mixing, oxygen produced by aeration equipment and natural diffusion is not dispersed throughout the water body and the efficiency of these processes (which are driven by tension differentials) is reduced. In daylight hr, thermal stratification and migration of motile phytoplankters to surface layers will tend to limit the banking of maximum oxygen reserves to counter nighttime respiration. Some progress is being made *in* deciphering the relative contribution of *water* column mixing and direct aeration to the overall oxygen *budget* in ponds using a combination of traditional aeration equipment (paddlewheels, aspirators, etc.) and low-speed submerged fans which put all of their energy into mixing.

The pond ecosystem is very efficient at assimilating and dispersing excess nutrients which would, otherwise, build up from the continuous feed inputs. A mass balance of nitrogen in intensive shrimp ponds indicates that 13 to 46% of nitrogen input via feed is lost through nitrification and atmospheric diffusion (Hopkins *et al.* 1993a). However, the importance Of aeration and mixing in N_2 diffusion and ammonia volatilization is Unknown. Ammonia volatilization rates are very low except when pH is high; pH is high only in the afternoon, after phytoplankton photosynthesis has stripped most of the CO_2 and produced large amounts of oxygen-the time when aeration is needed the least. The effect of afternoon aeration and mixing on afternoon pH and the effect of very high pH on the shrimp crop and other components of the pond ecosystem are also largely unknown.

From a global point of view, energy use may be the most important aspect of overall sustainability, particularly in light of recent predictions of fossil fuel supply limitations and the impact of greenhouse gases on the environment. Ironically, because it is a global issue, it has been difficult to obtain support on the state, or even national level, to pursue research on improving energy efficiency in shrimp farming.

PROTEIN TRANSFORMATIONS

The transformation of an inexpensive form of protein into an expensive form at a profit is the basis of much of today's commercial animal husbandry (e.g., poultry, swine, cattle feed lots). While such systems may be economically sustainable, they do not make the best use of the world's total protein resources. Like many forms of animal husbandry, intensive shrimp aquaculture is not very efficient in protein transformation. The edible portion of a shrimp may contain only 20% of the protein provided as feed (Hopkins *et al.* 1994). Some protein sources used in shrimp feeds are byproducts, not suitable for human nutrition (e.g., bone meal, feather meal, shrimp head meal, wheat middling). Use of these is encouraged, but inclusion of large amounts of such byproducts may increase the solid waste output of aquaculture operations if the feed formulation is not highly digestible with good feed conversion efficiency. Other feed components such as wheat flour and soybean meal can be processed for direct human consumption, and their use in shrimp feeds may be nutritionally inefficient for humans. The protein transformation issue is exacerbated by the use of varying amounts of fish meal in aquaculture diets. The primary source of fish meal is produced from harvesting wild stocks (e.g., U.S. Gulf and Atlantic menhaden, Peruvian anchovy) and these stocks are currently fully exploited.

There are several ways in which protein transformation efficiency can be improved in intensive shrimp culture, such as: (1) modifying the protein content and source in the diet to minimize the inclusion of protein from overexploited resources or from resources that could be used more effectively for direct human consumption; (2) development of culture technology which recycles waste produced *in situ* to create additional sources of nutrition for the crop; and (3) using multiple crops to generate overyielding without a proportional increase in feed input. or creating products which may be recycled into feed components.

A recent study indicates the protein content of feed used for intensive pond culture of marine shrimp can be reduced from 40% to 20% without affecting growth or production (Hopkins *et al.* 1995b). Additional work focused on reducing feed protein for intensive shrimp production is underway at institutions which are better equipped to address nutritional questions (Akiyama et al. 1992 and Aranyakananda and Lawrence 1993). However, it is well known that much of the shrimp growth can be attributed to their foraging on "naturally" occurring pond biota which, in turn, are nourished by nutrient wastes from formulated feeds (Hunter et al. 1987). Shrimp fed to excess in a clear-water tank system will typically grow at only about half the rate of cohorts receiving the same feed in an outdoor pond. Thus, while improvements in feed formulations will certainly increase protein transformation efficiencies, understanding and manipulating the pond environment to encourage waste recycling into a forage base has equally as much potential. There has been some preliminary work on methods to increase the biomass and composition of microbial-detrital floc through manipulation of carbon: nitrogen ratios, but without reliable results to date.

Pond water is rich in secondary biota which is sustained by the waste products of feed inputs for the principal crop. Metabolic waste products are principally dissolved inorganic ammonia and particulate fecal material which mineralizes to provide nutrients for growth of phytoplankton and bacteria which, in turn, nourish larger forms. As part of the work to reduce effluents, pond water at WMC was held over the winter after harvesting a shrimp crop. Water remaining in the pond through the fallow season developed a rich benthic community, an "aquatic pasture." The species composition will probably vary with the geographic region, but at WMC the aquatic pasture is dominated by several species of polychaete worms (*Capitella capitata* and *Polydura cornuta*) and an amphipod (*Gammarus mucronatus*), all of which are readily consumed by the subsequent crop of shrimp. The density of polychaetes and amphipods was 45,000/m^2 and 5,000/m^2, respectively. As the shrimp grew and their foraging pressure increased, the polychaete and amphipod populations were reduced and stabilized at less than 10/m^2 (Hopkins *et al.* 1995c). An adverse impact of the aquatic pasture, and perhaps the subsequent shrimp crop, was development of a large population of small grass shrimp (*Palaemonetes* sp.). While these small shrimp may be marketed for about US$2.25/kg, their overall financial impact has not been calculated. It is not yet known whether the grassshrimp can or should be effectively controlled by screening the water as it is pumped from one pond to another.

Different species (crops) will occupy different grazing niches, but unfortunately, complimentary combinations of species which may create overyielding effects in marine culture systems have been explored only superficially. Nevertheless, the combination of a fed crop (e.g., shrimp) and a filter-feeding bivalve seems particularly promising, as the nutrient input via feed creates a rich phytoplankton bloom which in turn, provides the food base for the bivalves. Combinations tested to date in South Carolina include shrimp polycultured with oysters (*Crassostrea virginica*), clams (*Mercenaria mercenaria*) (Hopkins *et al.* 1993b) and scallops (*Argopecten irradians concentricus*) (Walker et al. 1989). The combination of a fed crop of shrimp and a herbivorous or omnivorous fish is also appealing. Mullet (*Mugil cephalus*) have been successfully co-cultured in ponds containing shellfish and receiving shrimp pond effluent. Mullet were chosen because of their omnivorous feeding habits, a market for the flesh (albeit low price) and a lucrative market for the roe. While the mullet carrying capacity of such systems has not been determined, production levels as high as 4 metric tons/ha have been achieved and the fish matured with roe weights which equaled 10-30% of the whole weight for females. Nutrient-rich pond effluent also produced considerable amounts of seaweed (*Enteromorpha* sp.) which may have some market value, and is readily consumed by shrimp, or can be composted for use in terrestrial agriculture.

An important issue, peculiar to molluscan shellfish aquaculture, is the stringent shellfish sanitation regulations of the U.S. Food and Drug Administration (FDA), as administered by the respective state agencies. The WMC has recently completed a trial on the pond growout and marketing of clams. While the pond culture results were encouraging, the real significance of this work was approval of an Operating Plan whereby the product could be sold. The Operating Plan included sampling for fecal coliform and *Vibrio* spp. bacteria Overall, the concentration of fecal coliform bacteria and *Vibrio* spp. was lower in the pond than in the adjacent estuary from which water is drawn. This estuary is nearly pristine and is an approved shellfish gathering area. It is important to note that this pond was for clam monoculture only and an Operational Plan for clam/shrimp polyculture would not have been approved. However, as the FDA concerns are slowly addressed, and if the human health risks associated with pond-raised shellfish continue to appear inconsequential. FDA may become comfortable enough with pond growout to allow sale of molluscan shellfish from shrimp ponds.

Horizontal integration in production technology can be expanded to include terrestrial species (Ghate *et al.* 1994). The mode of action is the same in that waste products from one segment of the overall process are used as production inputs in another segment. Aquaculture sludge releases nitrogen slowly making it a good slow-release fertilizer and reduces migration of nitrogenous compounds into groundwater supplies. The WMC has made some preliminary observations of the effect of applying shrimp pond sludge accumulations on impoverished sandy Soil to in-

crease tilth, water retention capacity and fertility (Hopkins *et al.* 1994). In this study, sludge deposits were removed from an intensive shrimp pond weekly during the growing season. The total nitrogen removed with the sludge represented approximately two-thirds of the nitrogen added to the pond as feed. Preliminary results indicated that salts were quickly "washed" from the sludge by rainfall and sludge improved soil conditions and yield of a cover crop.

FUTURE DIRECTION

Over the next few yr, work on increasing shrimp farming sustainability at WMC will concentrate on (1) combining various factors which contribute to sustainability into comprehensive integrated systems; and (2) transferring this new-age technology to the commercial shrimp farms in South Carolina. A conceptual design of an integrated sustainable system which incorporates factors relating to land use, water use, energy use and protein transformations has been developed based on results of the studies described above (Sandifer and Hopkins, in press). This system incorporates complete water muse, oyster, mullet and shrimp polyculture, sludge removal, and sludge application on plots used to produce tomatoes and broccoli. The model prototype is based on expandable modules of four I ha ponds, one polyculture pond and the other shrimp monoculture ponds, plus associated sludge decanting and drying beds, a small inoculation pond, and a pump-piping system to move material within the system.

Finally, WMC is about to embark on a campaign to encourage shrimp farmers in the area to adopt the new technology. The WMC and the state extension network provide considerable support for the local industry including economic analysis, site selection and construction criteria, sources of equipment and supplies, day-to-day management assistance, and marketing advice. As a first step, farmers are being encouraged to dramatically reduce or eliminate water exchange. Feed manufacturers and the farmers are very interested in low-protein diets as such products make the feed manufacturer more competitive and reduce the farmers' expenses. Likewise, technology which reduces energy costs is sought for obvious reasons, both altruistic and economic. There is already considerable interest in polyculture of shrimp and other species as the Production costs for the secondary crops appear to be minimal.

The irony is that the shrimp farming industry in South Carolina is small and generally environmentally-benign. There is much less cause for concern in South Carolina than in countries where the size of the shrimp farming industry is several orders of magnitude higher and the industry is recognized as being unsustainable, even over the short-term (Hopkins and Sandifer 1995).

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Viral Diseases in Marine Aquaculture in Japan

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ABSTRACT

In Japan, marine aquaculture often has mass mortalities of cultured animals due to infectious diseases. This paper reviews diseases caused by viruses. These include viral diseases of marine cultured fish, viral diseases of shrimp and possible viral diseases of cultured abalone.

INTRODUCTION

Aquaculture has been rapidly developing and becoming more important in Japan. A diversity of species is seen in not only marine but freshwater aquaculture. Since there is a lack of seed fish, juvenile fish are often imported from foreign countries, especially from Southeast Asia. However, the rapid development of aquaculture has resulted in a problem, namely the occurrence of diseases including previously unknown diseases. This is caused by the intensification of fish culture and by recent entries of foreign pathogens. Among the pathogens, viruses are the most devastating infectious agents that afflict fish. The present paper reviews: (1) viral diseases of marine cultured fish, (2) viral diseases of cultured shrimp and (3) possible viral diseases of cultured abalone in Japan.

VIRAL DISEASES OF MARINE CULTURED FISH

Recently, several viral diseases have been reported in marine cultured fish. In some diseases, the causative agents have been observed by electron microscopy (EM) and isolated in established fish cell lines. In other diseases, however, the virus observed by EM could not be reproduced by cultured cells. Viruses contain one kind of nucleic acid (RNA or DNA) as their genome. The diseases caused by DNA viruses are shown in Table 1. These include lymphocystis disease, iridovirus infection of various marine fishes, viral epidermal hyperplasia (VEH) of Japanese flounder *Paralichthys olivaceus* and herpesvirus infection of coho salmon *Oncorhynchus kisutch*. The causative agents of lymphocystis disease and VEH have not been isolated using established fish cell lines.

Lymphocystis disease has occurred in several marine fish species, such as sea bass *Lateolabrax* sp., yellowtail *Seriola quinqueradiata*, *red* sea bream *Pagrus major* and Japanese flounder with the unsightly appearance of surface lesions (Miyazaki and Egusa 1972). Lymphocystis cells are observed mainly on the fins or body surface. Lymphocystis disease virus is an isometric particle with a large diameter and belongs to Iridoviridae.

Viral epidermal hyperplasia caused mass mortalities in larval and juvenile Japanese flounder (Iida *et al.* 1989). Hyperplasia was observed in the epidermal layer of fins and skin by histopathological methods. In addition, herpes virus particles were observed in the nucleus and cytoplasm of infected epidermal cells by EM.

Herpesvirus infection of maricultured coho salmon was first reported in Miyagi Prefecture in 1988 (Kumagai *et al.* 1994). Diseased fish showed excoriations of fin, erosion and ulcers on the body surface and pale spots in the liver. Necrosis of hepatocytes was the most evident histopathological change in the diseased fish. The isolated vi-

Table 1. Viral disease of marine cultured fish in Japan (DNA virus)

Disease	Virus Classification	Determination method Isolation	Electron	Host
Lymphocystis disease	Iridoviridae	—	0	Various marine fish
Red sea bream iridoviral disease (RSIVD)	Iridoviridae	0	0	Various marine fish
Viral epidermal hyperplasia (VEH)	Herpesviridae	—	0	Japanese flounder
Salmonid herpesvirus type 2 infection	Herpesviridae	0	0	Coho salmon

Table 2. Viral disease of marine cultured fish in Japan (RNA virus)

Disease	Virus Classification	Determination method		Host
		Isolation	Electron microscopy	
Yellowtail ascites virus (YAV) infection	Birnaviridae	0	0	Yellowtail
Viral deformity virus (VDV) infection	Bimaviridae	0	0	Yellowtail
Hirame birnavirus infection	Bimaviridae	0	0	Japanese flounder
Rhabdovirus infection	Rhabdoviridae	0	0	various marine fish
Virus nervous necrosis (VNN)	Nodaviridae	—	0	various marine fish

rus was identified as salmonid herpesvirus type 2 by morphological observation and serological tests.

An outbreak of the red sea bream iridoviral disease began in cultured red sea bream in Shikoku Island, Japan, in 1990. Since 1991, the disease has involved mass mortality of fish populations in the western part of Japan. Mass mortality has mainly occurred among red sea bream fingerlings. However, some mortalities of market-sized fish have also been recorded. The diseased fish were lethargic and showed severe anemia, petechiae of gills and enlargement of spleen (Inouye *et al.* 1993). The disease was histopathologically characterized by the development of enlarged cells deeply stained with Giemsa solution in the spleen, heart, kidney, liver and gills of infected fish. Inouye and co-workers also reported that the causative agent was a large, icosahedral, cytoplasmic DNA virus classified as a member of Iridoviridae (Table 1). The virus was tentatively designated as red sea bream iridovirus (RSIV). A similar type of disease resulting in serious damage occurred among several kinds of cultured marine fishes other than red sea bream, such as yellowtail. sea bass and Japanese parrotfish. The causative viruses, having characteristics of the family Iridoviridae and antigenically related to RSIV, were isolated from these fishes. Biological and physico-Chemical properties of the virus, production of monoclonal antibodies to RSIV and diagnosis method of theinfection were reported (Nakajima and Sorimachi 1994a, b, 1995).

RNA virus infections are shown in Table 2. These include yellowtail ascites virus (YAV) and related birnavirus infection of yellowtail, Japanese flounder, rhabdovirus infection of various marine fishes and viral nervous necrosis (VNN) of various marine fishes. The causative agents of these viruses have not been isolated using established fish cell lines.

In the early summer of 1983, an acute disease characterized by ascites occurred among cultured yellowtail fingerlings in the Seto Inland Sea, Japan. A bimavirus designated as YAV was identified as the causative agent of the disease (Sorimachi and Hara 1985). Epizootics generally occurred during May to June at water temperatures of 18-22°C. Fish which are less than 10 g in body weight are sensitive to this virus. The moribund fingerlings typically showed anemic gills, hemorrhage in the liver and severe ascites.

A new viral disease characterized by abnormal swimming behavior, deformity of the body and high mortalities occurred in yellowtail fingerlings at a hatchery in Kyushu Island, Japan (Nakajima et al. 1993). A birnavirus designated as viral deformity virus (VDV) belonging to the family Birnaviridae was identified as the causative agent of the disease. Advanced congestion in the liver, edema and anemia in the kidney and spleen, and congestion in various parts of the brain were observed in diseased fish.

Two birnaviruses, VDV and YAV isolated from cultured yellowtail were examined for their serological and biochemical properties (Nakajima and Sorimachi 1994a). Cross-neutralization studies showed that VDV was closely related to YAV but clearly distinct from the three type strains of infectious pancreatic necrosis virus (IPNV). VDV and YAV contained two genome segments and mobility of the smaller segment showed a slight difference between the two. On the other hand, polypeptide electropherotypes showed a clear difference between VDV and YAV. The Production of MAbs against YAV or VDV was reported (Nakajima and Sorimachi 1996a, b).

A birnavirus infection was also reported in juveniles of the Japanese flounder (Kusuda et *al.* 1989).

Rhabdovirus infection of Japanese flounder first occurred in 1984. The causative virus was designated hirame rhabdovirus (HRV, Kimura et al. 1986). The signs of the disease are congestion of the gonads, focal hemorrhaging in skeletal muscle and fins, and the accumulation of as-

Table 3. Viral disease of marine cultured fish in Japan

Disease	Virus Classification	Determination method		Host
		Isolation	Electron microscopy	
Erythrocytic inclusion body syndrome (EIBS)	Togaviridae?	—	0	Coho salmon
Kuchijiro-shou (snout ulcer disease)	?	0	0	Tiger puffer

Table 4. Viral disease of shrimp in Japan

Disease	Virus Classification	Determination method		Host
		Isolation	Electron microscopy	
Bacuoloviral midgut gland necrosis (BMNV)	?	—	0	kuruma shrimp
Penaeid acute viremia (PAV)	?	—	0	kuruma shrimp

citic fluid. HRV was isolated from ayu *Plecoglossus altivelis*, black sea bream *Milio macrocephalus* and from mebaru *Sebastes inermis*.

Viral nervous necrosis has been reported and caused high mortalities in hatchery-reared larvae and juveniles of marine fishes in Japan, such as Japanese parrotfish. redspotted grouper *Epinephelus akaara,* striped *jack Pseudocaranx dentex,* Japanese flounder, tiger puffer *Takifugu rubripes*, kelp grouper *Epinepholus moara and* bar-fin flounder *Verasper moseri* (Yoshikoshi and Inouye 1990, Mori et al. *1991,* Arimoto et *al, 1993,* Nakai *et al.* 1994, Nguyen et al. 1994). Vacuolation in retinal and brain tissue is characterized by histopathological observation (Mori *et al.* 199 1). The causative viruses are unenveloped, round-shaped virions, approximately 25-30 nm in diameter which belong to Nodaviridae (Mori *et al.* **1992).**

Erythrocytic inclusion body syndrome (EIBS) is a serious viral disease of salmonid fish. Epizootics attributed to EIBS occurred among populations of coho salmon cultured in seawater in Japan (Takahashi et *al.* 1992). The signs of the moribund fish were severe anemia and yellowish livers. Characteristic inclusion bodies in erythrocytes contained enveloped viral particles with a diameter of approximately 77 nm. However, the causative viral agent has not been isolated using established fish cell lines and the virus seems most likely to be a member of the family Togaviridae (Arakawa et *al.* 1989).

In Kuchijiro-shou (snout ulcer disease) of tiger puffer, diseased fish showed necrosis around the mouth andaggressive biting behavior. Viral particles were observed in the brain of the diseased fish by EM.The causative virus was isolated in cultured cells and has not yet been classified (Inouye *et al,* 1992, Table 3).

VIRAL DISEASES OF SHRIMP IN JAPAN

The viral diseases of kuruma shrimp are summarized in Table 4. These include baculoviral midgut gland necrosis (BMN) and penaeid acute viremia (PAV) which had tentatively been called RV-PJ infection. BMN of kuruma shrimp larvae has occurred since 1971 in shrimp-culture farms and in seed-production sections and since then it has often caused mortalities of 90% during the mass production of kuruma shrimp larvae in Japan (Momoyama 198 1). The midgut gland and the intestine are affected by the virus. The properties of BMNV and cloning of the genomic DNA were reported by Arimoto *et al.* (1995). The virus has not been isolated using established cell lines. A diagnosis of BMNV is the observation of hypertrophy in the midgut glands (Momoyama 1983).

In 1993, a new viral disease with high mortality occurred among kuruma shrimp at farms in the western part of Japan (Inouye *et al.* 1994, Nakano *et al.* 1994). Red coloration or discoloration and white spots on the body were characteristic signs of diseased shrimp. Degenerated cells were observed in various tissues originating from the meso- and ectoderm (Momoyama *et al.* 1994). EM revealed a rod-shaped, enveloped, nonoccluded virus in the nuclei in the cuticular epidermis of the stomach or the lymphoid organ. However, the virus differs from any kind of viruses reported from crustaceans in morphology,size, site of assembly of virions in host cells, and histopathology. The virus was tentatively designated penaeid rod-shaped

DNA virus (PRDV). The name of the disease was Proposed as PAV (Inouye *et al.* 1996). The classification of the virus is now under investigation.

POSSIBLE VIRAL DISEASE OF ABALONE IN JAPAN

A fatal disease with muscular atrophy has often occurred in juvenile abalone Nordotis discus at various hacheries in Japan (Nakatsugawa *et al.* 1988). The causative agent has not been identified. The disease is characterized by the formation of tumors in the pleuropedal neurotrunk. Although isolation of the causative agent by the use of several fish cell lines was not successful, the disease was transmitted with a 0.22 μm filtrate of diseased abalone homogenate (Nakatsugawa 1990). These results show that the disease is infectious and the causative agent may be a virus.

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Effects of Cultured Fish Feces on Algae Growth

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ABSTRACT

In order to understand the role of fish feces in material cycling in aquaculture grounds, the effects of cultured fish feces to algal growth rates were examined by feeding probiotic supplements under laboratory conditions. Four 1000-L round-shaped plastic tanks were employed for culturing red sea bream, ***Pagrus major*** (average BL=12.5 cm). Twenty fish were reared in each tank with a running water system. The fish in tanks A, B and C were fed on moist pellets with 1% of ***Ulva pertusa*** fragments, 0.1% of yogurts and 0.5% of ***U. pertusa*** fragments and 0.05% of yogurts, respectively. The fish in tank D were fed on dry pellets alone. The feces were collected once a day and fermented by a rotator for 14 days. Algal growth potential (AGP) was determined for the culture of ***Nannochloropsis oculata***. **The algae** were inoculated by fecal medium which was extracted after fermentation in an incubator. The temperature and light conditions in the experiments were adjusted to 23°C, 7500 lux and 12L:12D. The feces extracts collected from tanks A, B and C showed higher AGP in each trial. The feces collected from tank D presented always lower AGP. The highest population density of N. *oculata*, 34.8 x 10^6 cells/ml, was observed in tank A which was fed moist pellets with 1% of ***U. pertusa*** fragments.

INTRODUCTION

The conversion rates of food to fish are well studied in aquaculture. From the viewpoint of aquaculture ecology, however, bioconversion of fish to feces, or bioconversion of feces to algae is as important as the bioconversion of food to fish. In order to understand the role of fish feces in aquaculture farms, the effects of cultured fish feces to algal growth rates were examined by feeding probiotic supplements in laboratory conditions. In the present experiments, effects of cultured fish feces to algal growth were examined.

MATERIALS AND METHODS

The fish culture experiments were conducted in the Azumacho Fish Seedling Center in November 1994. Four 1000 L round-shaped plastic tanks, A, B, C and D, were employed for culture of red sea bream, ***Pagrus major*** (average BL=12.5 cm). Twenty fish were reared in each tank with a running water supply system. The fish in tanks A, B, and C were fed on moist pellets with 1% of *Ulva pertusa* fragments, 0.1% of yogurts, and 0.5% of ***U. pertusa*** fragments and 0.05% of yogurts, respectively. The fish in tank D were fed on dry pellets alone. The sterile ***U. pertusa*** were cultured in the fish farm around the center and were minced by the specially designed device for moist pellets. The yogurts containing multispecies of the lactate-fermenting bacteria, *Lactobacillus* ***bulgaricus*** and ***Streptococcus thermophilus***, were obtained daily from a local food store. The fish were satiated in the morning. The feces were collected in the evening. The feces were fermented by a rotator (8 rpm) for 14 days under 23°C. Algal growth potential (AGP) of this fecal medium was determined by using culture of ***Nannochloropsis oculata***. The culture experiments of N. *oculata* were conducted in an incubator adjusted to 23°C, 7500 lux and 12L:12D. The AGP tests were repeated seven times in the Faculty of Agriculture, Kinki University, during January and March 1995. The maximum population density was used as AGP throughout the culture experiments. At the end of the experiments, water qualities such as NH_4-N, NO_2-N, NO_3-N and PO_4-P in the fecal medium were measured

RESULTS AND DISCUSSION

The results which showed relatively higher AGP throughout the experiments are presented in Pigs. 1 and 2. In trial 2, the U-Y mix group showed a higher population density of 86.6 million cells/ml. The population densities in ***Ulva, yogurts*** and DP groups were 68.5, 48.3 and 42.2 million cells/ml, respectively. In trial 4, the ***Ulva*-supplemented** group showed the highest population densities through all the trials as 93.4 million cells/ml (Pig. 2). The densities in the U-Y mix, yogurts and DP groups in trial 4 were 88.6, 76.6 and 30.0 million cells/ml, respectively. The results obtained in each trial are summarized in Table 1 and Fig. 3. The highest population density was found in

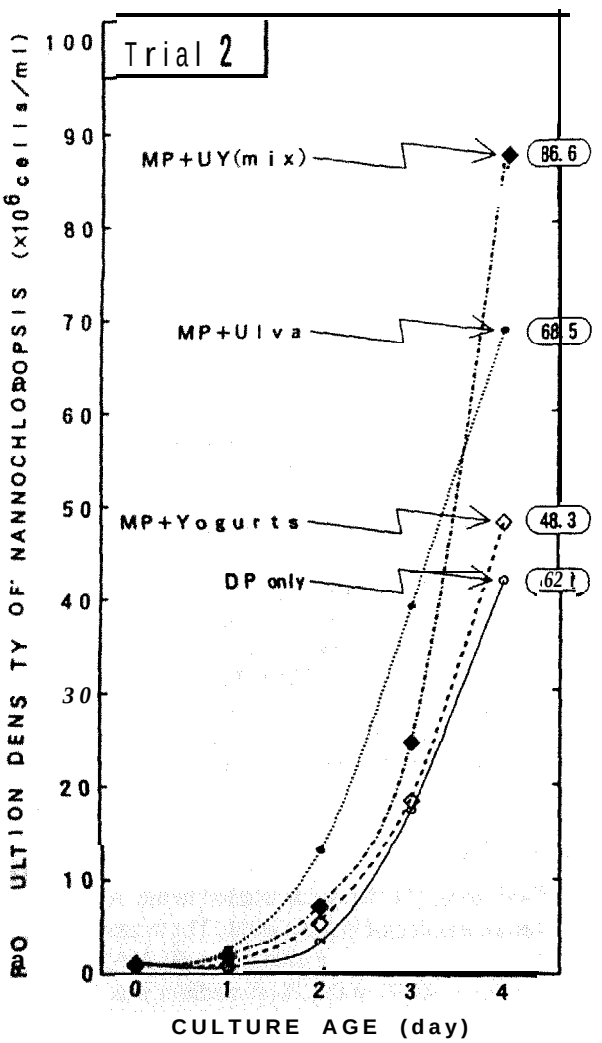

Fig. 1. Population growth of Nannochloropsis oculata *cultured by the fecal medium obtained from red sea bream fed on pmbiotic supplements (2nd trial).*

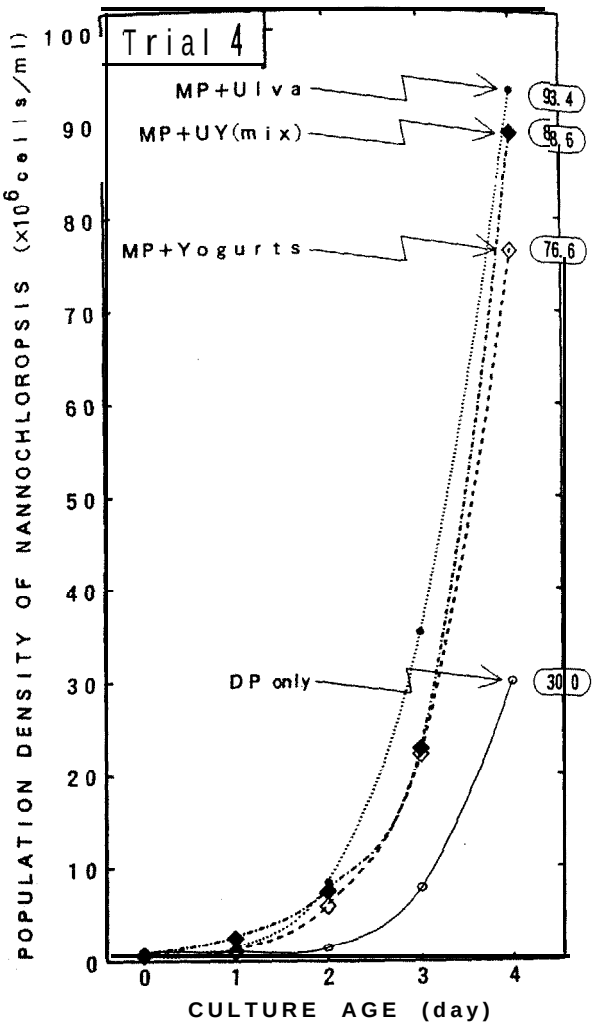

Fig. 2. Population growth of N. oculata *cultured by the fecal medium obtained from red sea bream fed by pmbiotic supplements. (4th trial)*

the U-Y mix group which showed 71.4 million cells/ml an average. The second highest density, 68.5 million cells/ml, was observed in the *Ulva* group. The third measurement was yogurts. The final fourth one was observed in the DP group. The DP group showed lower growth rates throughout the experiments.

Table 2 shows the results of water quality analysis observed at the end of the experiments. IN/IP ratios in the yogurts, *Ulva*, U-Y mix and DP groups were 9.9, 15.2, 72.5 and 190.5, respectively. The ratio in the DPgroup was remarkably high. It may be concluded that the higher IN/IP ratios caused lower algal growth rates.

Higher algal growth rates should reflect the fast material recyclings in the waters. When the fish were fed on *Ulva* and/or yogurts, each group showed higher algal growth rates. Therefore, those probiotic treatments are effective for fast material cyclings in the waters. This system could be termed an environmentally friendly culture method. Recently, the sustainable aquaculture systems based on the homeostasis of ecosystems have been studied by several biologists (Ackefors 1990, Hirata 1989, Lee 1995). The problem is how to promote the energy flow in the sea farms. Nagahama and Hirata (1989), Xu and Hirata (1990), Hirata et al. (1994), Yamauchi et *al.* (1995), and Matsuda et al. (1996) have studied the feedback culture system by the polyculture of *Ulva* and red sea bream and/or Japanese flounder, *Paralichthys olivaceus*. According to their reports, when the fish were fed back on 2% Of the sterile *Ulva* per diet, the growth rates increased 1.5%, with higher survival rates. Dissolved oxygen contents in the polyculture cage increased 9%. The carbon dioxide in the cage decreased 4%. The *Ulva* group in the present experiment reconfirms the results obtained in the reports meationed above.

Probiotic Culture systems have been developed in the fields Of animal husbandry (Fukushima and Nakano, 1995).

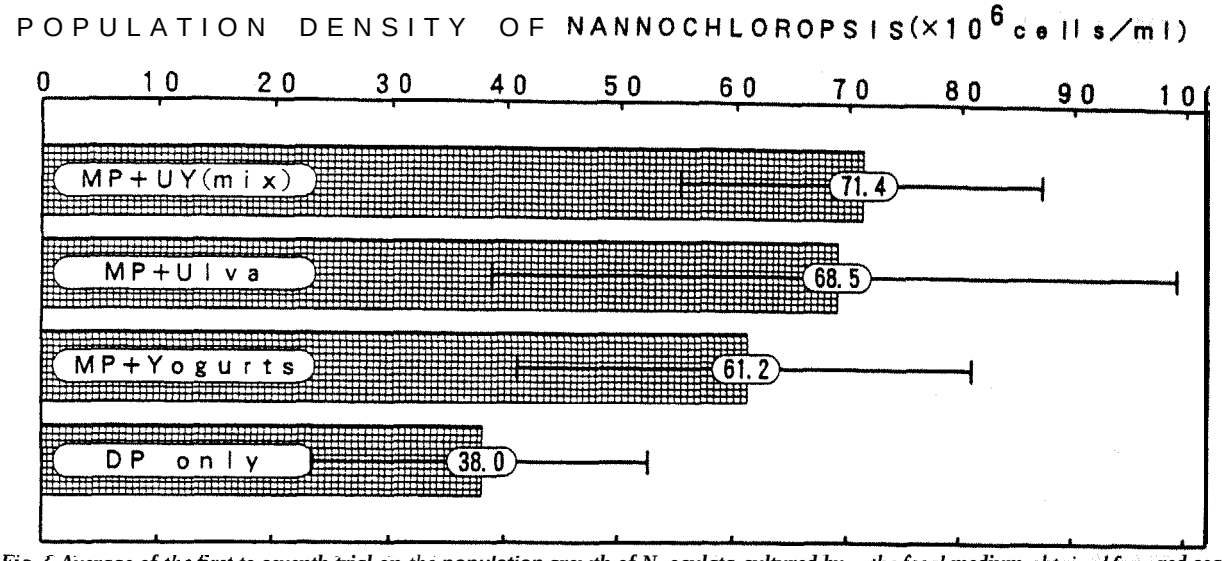

Fig. 3. Average of the first to seventh trial on the population growth of N. oculata cultured by the fecal medium obtained from red sea bream fed on probiotic supplements.

Table 1. Algal growth rates of *Nannochloropsis oculata* in each trial. (MP: Moist Pellets, DP: Dry Pellets, U: Ulva, Y: Yogurts)

	MP+Ulva	MP+Yogurts	MP+UY (mix)	DP only
		(x106 cells/ m/)		
Trial 1	41.2	44.8	53.0	
Trial 2	68.5	48.3	86.6	42.2
Trial 3	66.0	63.0	79.3	48.5
Trial 4	93.4	76.6	88.6	30.0
Trial 5	42.2	45.6	46.2	25.4
Trial 6	33.6	44.9		17.7
Trial 7	134.8	105.3	14.7	64.0
Average	68.5	61.2	71.4	38.0
S D	33.1	21.1	16.2	15.5

Table 2. Inorganic nitrogen and phosphate contents in the fermented fecal medium. (IN: Inorganic Nitrogen, IP: Inorganic Phosphate)

	NHCN	NO2-N	NO3-N	PO4-P	IN/IP
		(µg—at/l)			
Yogurts	**5049**	**tri.**	**tli.**	**509**	**9. 9**
Ulva	17909	**tri.**	tli.	1178	15. 2
UY (mix)	24735	**tri.**	tri.	341	72. **5**
Control	48589	**tri.**	tri.	25.5	190. 5

In the case of aquaculture, however, the probiotic research field has just been initiated. The yogurt supplements in this experiment could possibly be applied to the probiotic techniques in aquaculture.

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Monitoring Systems Useful in Mass Production of Larvae for Japanese Fish Culture

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ABSTRACT

Recognizing a need for quantitative and labor-saving management in fish culture technology, we describe monitoring systems actually in use for continuous measurements in mass production in Japan, with special reference to two systems for water quality regulation: first, a basic system of independent and centralized measurement types for selected water quality parameters (temperature, hydrogen-ion concentration, and dissolved oxygen), and, second, a system for regulating the phytoplankton culture medium. Chemical and optical sensors contribute together to the functioning of these systems. Effective integration of these newer monitoring methods with empirical ones now in use should be me next step in automated monitoring.

INTRODUCTION

The mass production of fish fry has expanded in Japan to reach a level of more than one million fry a year in most of the fish culture facilities which rear such species as the red sea bream, *Pagrus major,* and the Japanese flounder, *Paralichthys olivaceus. Nevertheless,* such mass production has not been easy, because of some difficulties in controlling the process, even if the latest technology was used. This problem has indicated a need for a focus on establishing in these facilities monitoring systems for continuous measurement with quantitative and labor-saving capabilities.

We here describe the monitoring systems actually in use at related facilities in Japan, with special reference to two models for water quality regulation-one effective for basic parameters of water quality and the other for medium regulation in phytoplankton culture.

BASIC WATER QUALITY MONITORING SYSTEM

MONITORED SUBJECTS

Fish farming facilities now use continuous measurement of temperature, hydrogen-ion concentration (pH), and dissolved oxygen (DO) for water regulation in culture tanks, especially for brood stocks and larval breeding. In those continuous measurements, water temperature is always fundamental, and it has been measured for a longer time period than the other parameters. Recently, the other two parameters—pH and DO--have become popular items for continuous measurement. Earlier, Fujita et al. (1982) developed a continuous measuring system ofwater quality, including temperature, pH, and DO as well as flow rate in the fish fry tanks at the Kagoshima Prefectural Fish Farming Center, Tarumizu. They did not, however, emphasize the importance of other parameters, such as salinity and turbidity.

MODEL TYPES AND DESIGN

Up-to-date models of the continuous measurement system may be classified into the following-types 1 and 2—from the merit-rating viewpoint. The type 1 system consists of three parts (Fig.1): sensor. monitor, and receptor or computer parts. The sensor part is composed of a set of sensors and their terminal devices. Every tank to be measured is furnished with the set. The terminal device converts sensor signals, and transmits them at 4-20 mA to the monitor. This part may receive sensor signals from a maximum of 16 terminals, display available measurements, convert analog signals to digital ones, and transmit these data to the receptor. This final part is a personal computer system which on the one hand displays on the cathode-ray tube necessary information, that is, measurement-related graphs, indicated alarms, and daily and monthly reports, and on the other hand stores available data. Alarm signals are automatically reported from the receptor to programmed addresses through an emergency warning device.

This type of system is rather simple to assemble and it provides continuous data at selected intervals. Another advantage of this type of system is its compensability. If any part of the sensor fails and does not transmit signals, other component parts may transmit an effective amount of signals. In fact, the most accepted system in Japan is this type.

If we consider the type 1 system independent in the disposition of sensors, the type 2 system (Fig. 2) provides a centralized disposition of sensors. The type 2 system consists also of three parts: sampling, sensor-monitor, and receptor. Sample water is conveyed for measurement from

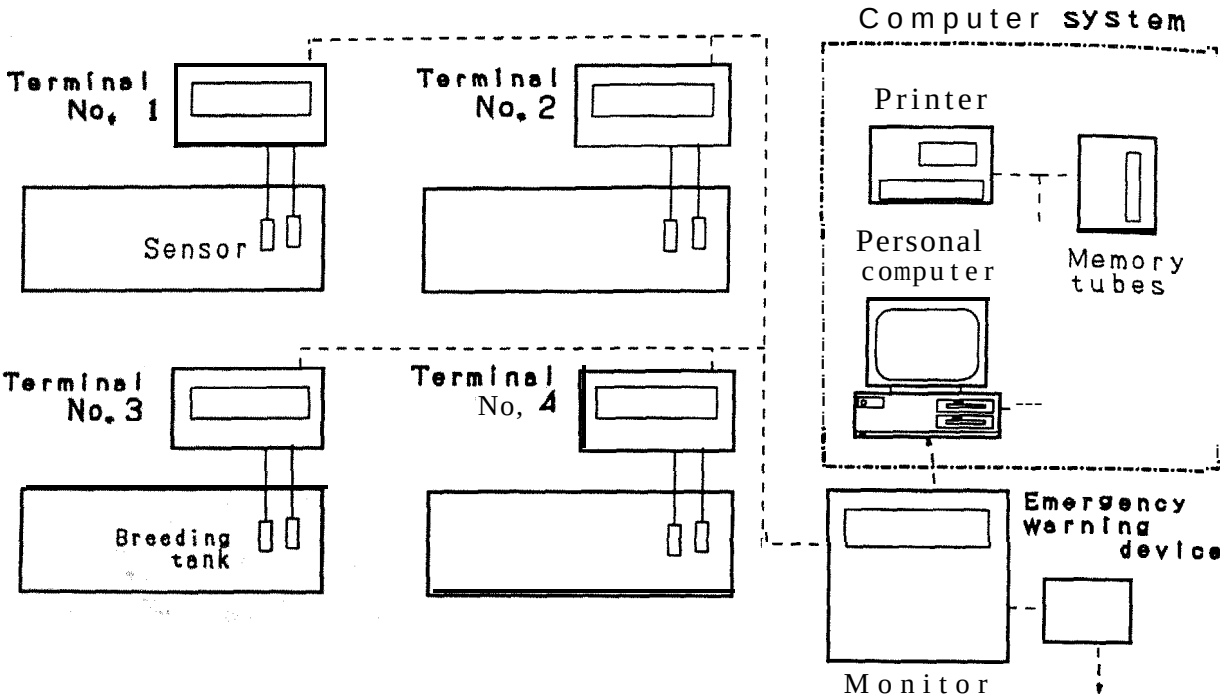

Fig. 1. Composition of the monitoring system model type 1.

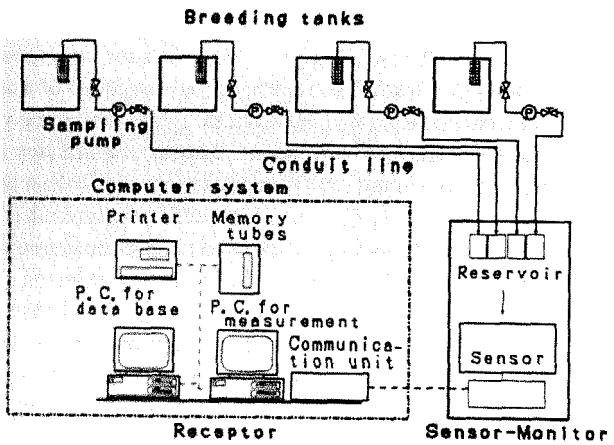

Fig. 2. Composition of the monitoring *system model type 2.*

a group of tanks up to the sensor-monitor by a pumping apparatus attached to each tank through a conduit line. The sensor-monitor is regulated to accept the sample water into a reservoir at intervals from the respective tanks for measurement. As soon as the reservoir is filled with water, the water flows down automatically into the succeeding tank with a set of sensors for common use. The set is composed of sensors which measure temperature, pH and DO, as well as some optical parameters of water quality. This optical sensor unit will be illustrated in detail later. Additional functions of this part of the system may be (1) automatic cleaning of the tank containing the sensors, and (2) an alarm setting for regulation of given parameters within the programmed range. Available data from this part are converted from analog to digital signals, and transmitted to thereceptor through a communication apparatus. The final part, or a computer system, stores and controls continuous measurements and other necessary data as a functional data base for breeding management.

The type 2 system offers some advantages; first, it economizes on sensors which may be very expensive, and reduces professional maintenance, such as regulation of delicate sensor units; second, it easily gives higher precision to the data, because each parameter is measured in this system by a single sensor unit, and most of the measurement errors among different units may be eliminated.

Kanamaki and Shirojo (1994) tried to develop a type 2 model (Fig. 3), applying an up-to-date sensor unit of optics, under the following circumstances: during early stages of fish breeding, phytoplankton species of Nannochloropsis are usually added to the rearing water to feed therotifers as well as to improve water quality. Concerning the feeding of the animal, inspectors determine when and how much phytoplankton should be added. Too much may cause a persistence of prey organisms in the water; this may result in a sudden deterioration of water quality by rapid mortality of the prey organisms. To regulate the addition of phytoplankton, concentrations may be controlled on the basis of numerical data provided as relative fluorescence intensity by a sensor for chlorophyll a with an excitation wavelength of 436 nm and an emission wavelength of 685 nm. This sensor may be used to measure detritus concentrations in combination with a beam transmittance sensor for near infrared rays.

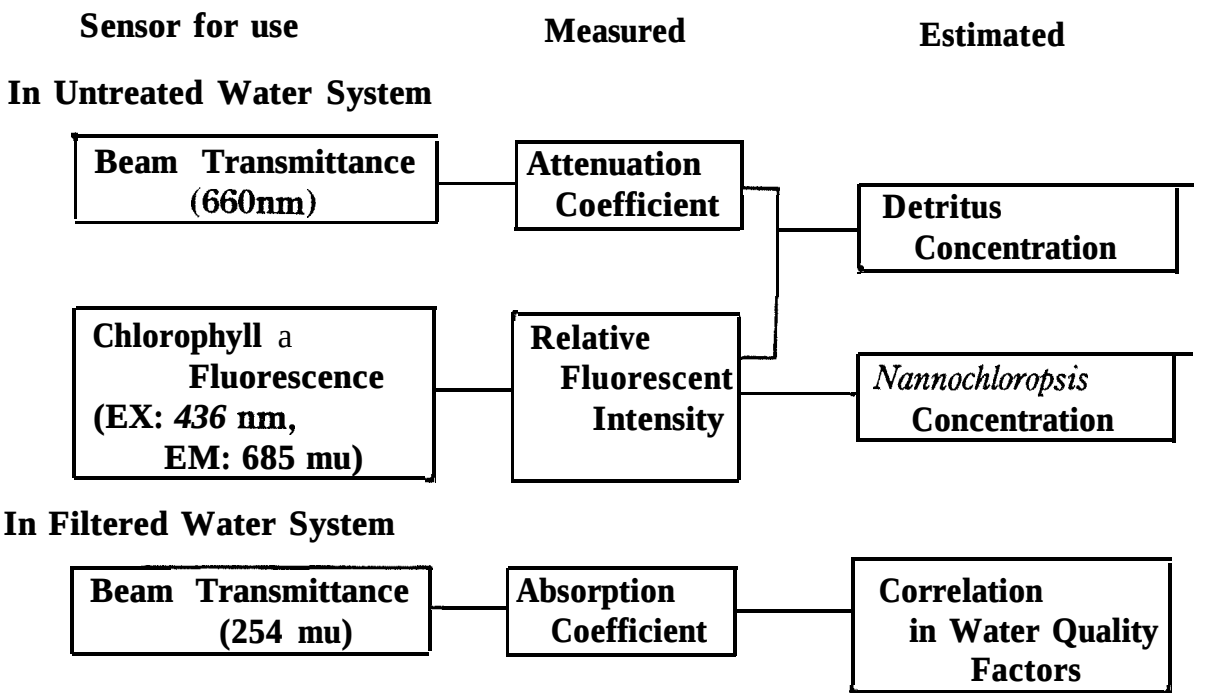

Fig. 3. Flow diagmm of water quality monitoring by opticalmeasurement. Columns: left, sensor series: middle, measured properties; right, estimated properties. EX: excitation wavelength; EM: emission wavelength (Kanamaki andShirojo 1994).

During the period of larval breeding in standing water, the feeding of the rotifers eventually reduces water quality in the breeding tank. Preventive measures for this trend are to exchange water appropriately just after the period when larvae grow enough to adapt to a stronger flow during water exchange, and just before a lethal deterioration of water quality. This larval breeding management technique has previously depended upon the empirical estimation of the technicians in charge.

As for the water exchange in larval breeding, numerical management is useful for this maintenance, depending upon continuous measurement of nitrogen in the form of ammonium ion (NH_4-N), and chemical oxygen demand (COD) in a dissolutive condition. Dissolved matter may be detected through the absorption coefficient of ultraviolet rays in filtered water. This coefficient is measured by a beam transmittance sensor. Water pollution in the rearing water then may be indicated by the absorption coefficient of ultraviolet rays in relation to the concentration of the dissolved matter.

In the field of marine science, optical sensors have been developed especially for oceanographic observation and for biotechnological management in fish culture, and have become available in small-sized and moderate-priced models. Similar models could be easily applied to water quality monitoring in aquaculture operations. The following are important current models: (i) a fluorescenceintensity sensor (Fig. 4) designed by the Fuyo Ocean Development and Engineering Co., Ltd., Tokyo, Japan, especially for continuous measurement in a high density; its size is 30 cm in diameter and 45 cm in length; (ii) a smaller sensor (Fig. 5) with the same function as above for oceanographic observation, designed by the ALEC Electronics Co., Ltd., Kobe, Japan; (iii) a sensor (Fig. 6) of the same function and purpose as above, designed by the Western Environment Technology Laboratories, Inc., Philomath,

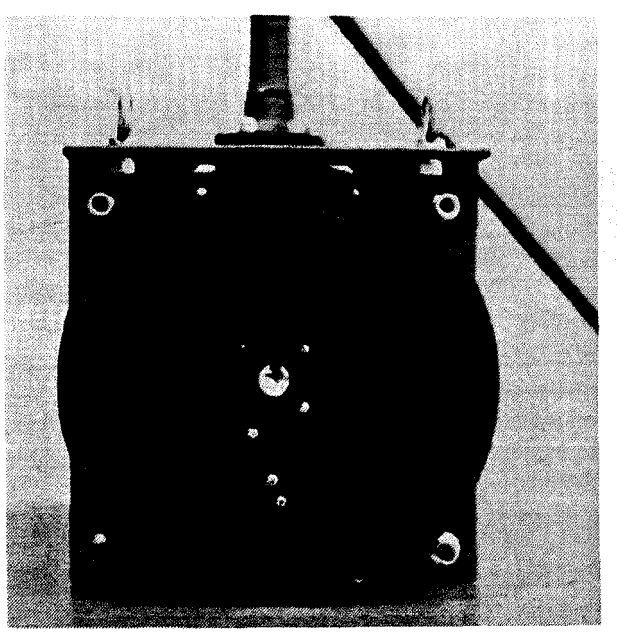

Fig. 4. A fluorescence intensity sensor unit for continuous measurement. A wiper is provided to keep clean its light-slanting window at the center of the body.

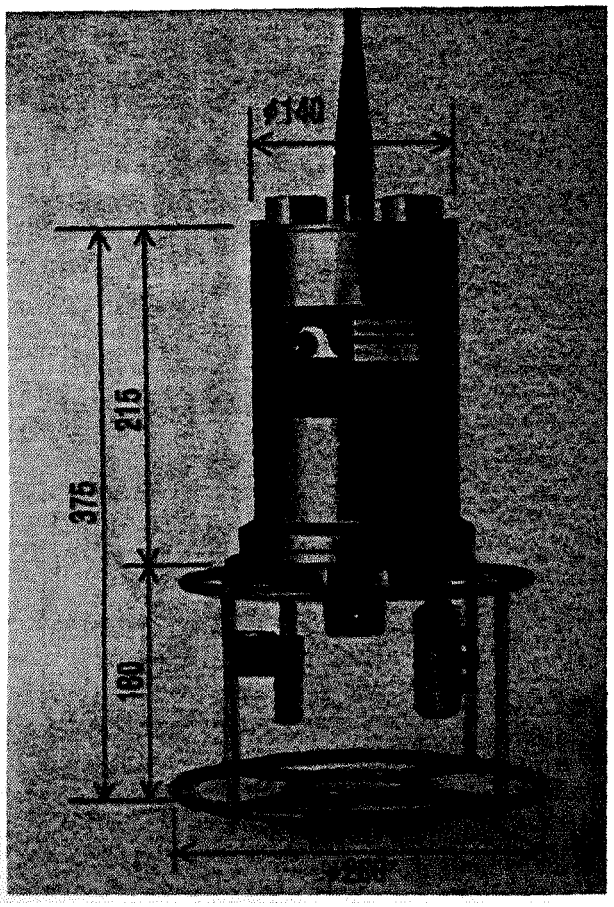

Fig. 5. A fluorescence intensity measuring instrument for oceanographic observation. Sensor assemblage: at the foot part (from left to right), sensor for salinity, fluorescence intensity, and turbidity; inside the body, sensors far water temperature and depth (catalog of the LEC Electronic Co., Ltd., Kobe, Japan).

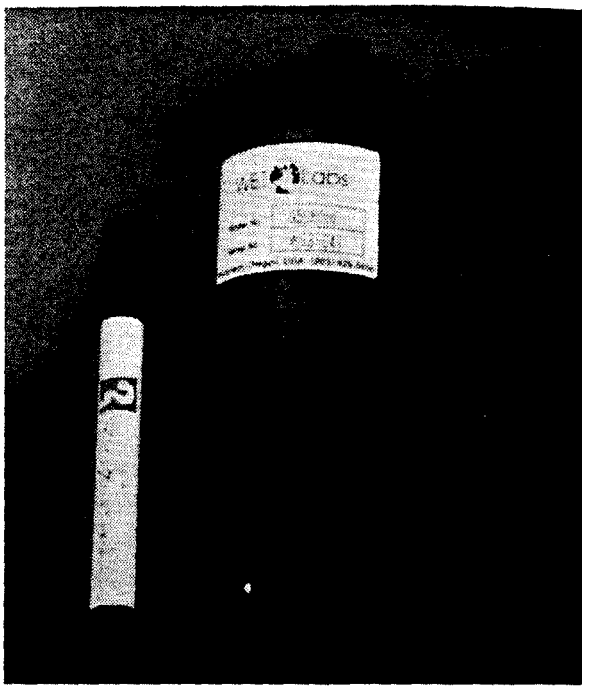

Fig. 6. A smaller fluorescence intensity probe (right) for oceanographic observation (catalog of CT and C Co., Ltd., Tokyo, pan).

A turbidometer unit for monitoring the ceil number of microorganisms. Parts assemblage (from left to right): controller and a set of probes (high-temperature-resisting-type and standard one) (catalog of the Automatic System Research Co., Ltd., Tokyo, pan).

OR, USA; (iv) a turbidometer (Fig. 7) with scattering and transmittance light measurement functions, designed by the Automatic System Research Co., Ltd., Tokyo, Japan, to monitor the cell numbers of cultured microorganisms (Yamane 1993).

MATHEMATICAL BASIS FOR MONITORING ALGAL DENSITIES

To estimate the concentration of *Nannochloropsis* species of detritus, the following series of equations is applicable.

The relationship between *Nannochloropsis* density (N) and fluorescence intensity (F) is given in equation (1), and total attenuation coefficient in equation (2) as a function of N and detritus concentration (D).

$$F = \alpha N \qquad (1)$$

where a is a proportional coefficient.

$$(c\text{-}c_w)\lambda = \beta_\lambda N + \gamma_\lambda D \qquad (2)$$

where c is total attenuation coefficient of culture water;

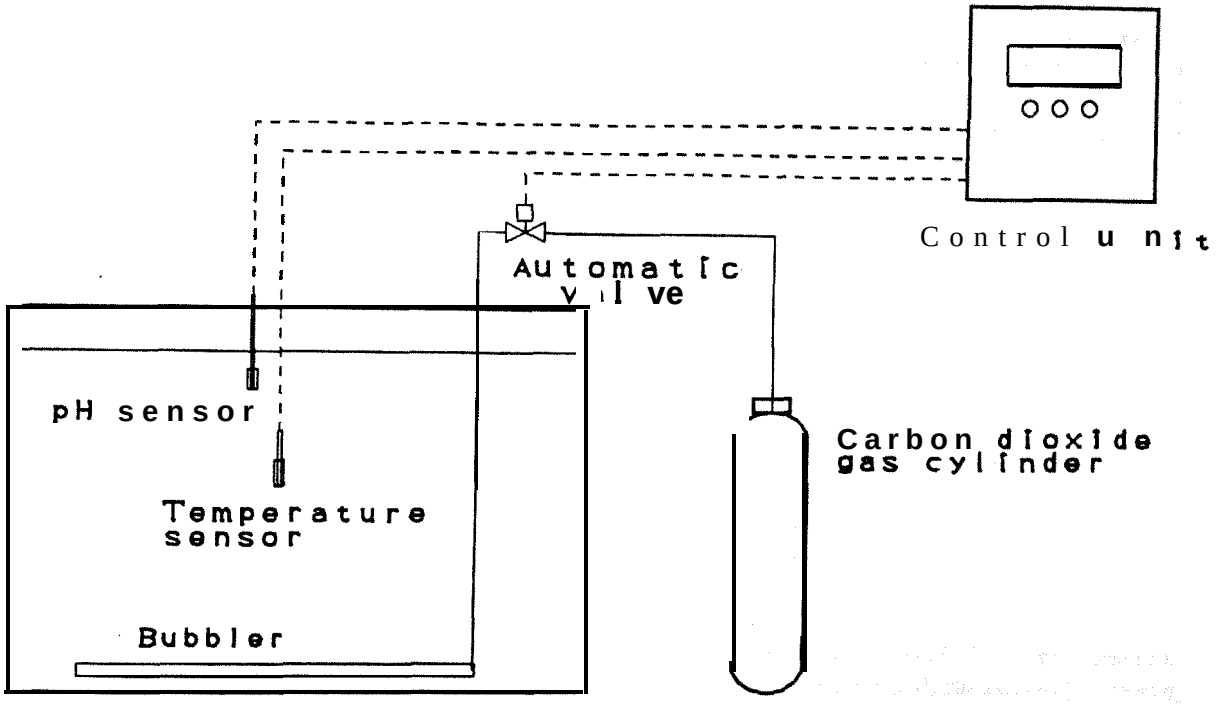

Fig. 8. A forced gas feed system composition for high density culture of phytoplankton.

c_w, total attenuation coefficient of pure water; λ, wavelength; β, γ, proportional coefficients.

In these equations, the terms for dissolved matter and rotifers are negligible. In these interrelationships, the equation (1) gives the fluctuation of the plankton density. This is derived by measuring fluorescence intensity, F, because α may be previously given on a working curve of F and N. The detritus concentration, D, is given from the interrelationships shown in equations (1) and (2); the elimination of N in these expressions gives equation (3), or an interrelationship between F and the left side term $(c - c_w)\lambda$.

$$(c - c_w)_\lambda = \frac{\beta}{\alpha} F + \gamma D \qquad (3)$$

These equations may give total attenuation coefficient, γD, continuously in relation to D, because either c or F is a continuous measurement.

MEDIUM REGULATING SYSTEM FOR PHYTOPLANKTON CULTURE METHODOLOGY

The second monitor system introduced here is a management system for phytoplankton culture through regulation of the culture medium. This culture is well known as a basic requirement for feeding of fish larvae. Recent efforts have been aimed at establishing a high density and stable culture of nutritive phytoplankton by adding carbon dioxide gas into the culture medium, in order to control photosynthesis, Hamasaki and Maruyarna (1991) studied the effect of carbon dioxide gas added to *Nannochloropsis* culture, and they suggested that it is useful to add the gas into the culture medium especially during a high water temperature period. An adequate solution has not been found, however, for the problem of how to obtain better effects from the gas supplement and how to regulate such a treatment appropriately, although feasibility studies have been started already on fish seed production by highly motivated researchers (Osawa and Nagano 1994).

As for our experimental application (Fig. 8), the gas supplement is regulated by an on-off control in relation to the pH level in the culture tank containing sensors for temperature and pH. As another aspect of our experiment, we used an optical device, or beam transmittance sensor of near infrared rays, to measure continuously the density of cultured phytoplankton. Available data are derived from the transmittance of a parallel ray pencil of 690 nm in the culture water. The transmittance can be converted for suitable analysis to total attenuation coefficient as given in equation (4).

$$(c - c_w)_\lambda = \frac{1}{L} \ln\left(K_\lambda \frac{I_a}{I_w}\right) \qquad (4)$$

where L is the path length; K_λ, a correction factor; I_w, value in the water; I_a, value in the air.

In the case of the 690 run wavelength light, optical absorption by dissolved matter is so little as to be negligible in the culture medium. The total attenuation coefficient concerned with the phytoplankton, c'_{690}, may be given by equation (5).

$$c'_{690} = c_{690} - c_{b\,690} \quad (5)$$

where the background estimate, $c_{b\,690}$, is deducted. The total attenuation coefficient, c'_{690}, may fluctuate in proportion to the density and projective area of planktonic cells as shown in equation (6),

$$C'_{690} = \Sigma\, \Omega_{690}\, n\, A \quad (6)$$

where Ω_{690} is efficiency factor: n, density in number of *Nannochloropsis* species per unit volume; A, projective area of the cells.

Consequently, at alight intensity period when cells do not increase in number, fluctuation of the phytoplankton growth apparently depends on the projective area, in close relation tdo the ceil growth in diameter. On the contrary, considering the behavior of daily periodical measurements, the projective area is almost inactive at every daily measurement. This means that the coefficient is affected exclusively by the cell numbers in this case. Based on equation (7), the specific growth rate, μ, is optically estimated with the aid of c'_{690}, or continuous measurements of the total attenuation coefficient in relation to the phytoplankton growth,

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu F \quad (7)$$

where F = 1 in the exponential growth phase; F = exp $\{-\delta (t - t_d)\}$ in the decreasing growth phase; μ, specific growth rate in the exponential growth phase; 6, decreasing growth factor; t_d, days elapsed before the decreasing growth phase.

As referred to already, the variable c'_{690} is proportional to the phytoplankton cell numbers. Therefore, equation (8) can be formed for the growth curve in the exponential growth phase, and in this case the rate is regarded as constant:

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu \quad (8)$$

In the decreasing growth phase, equation (9) indicates that the rate decreases exponentially with the passage of time.

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu \exp\{-\delta (t - t_d)\} \quad (9)$$

The change seen in the phytoplankton cell numbers may be deduced by applying this equation to the growth process of the organisms.

AN APPLICATION

In some of our experiments with synchronized culture for Nannochloropsis, fluctuations in specific growth rates seem to reveal an interesting fact (Fig. 9). In those cases, the trend is apparently divided into two phases: a stable or constant phase and a straightly decreasing one. The former phase corresponds to the change in the exponential growth phase referred to in the equation mentioned above, and the latter one corresponds to the change in the decreasing growth phase in the same case. In other words, the equation, especially in the latter phase, approximates effectively the phenomena concerned.

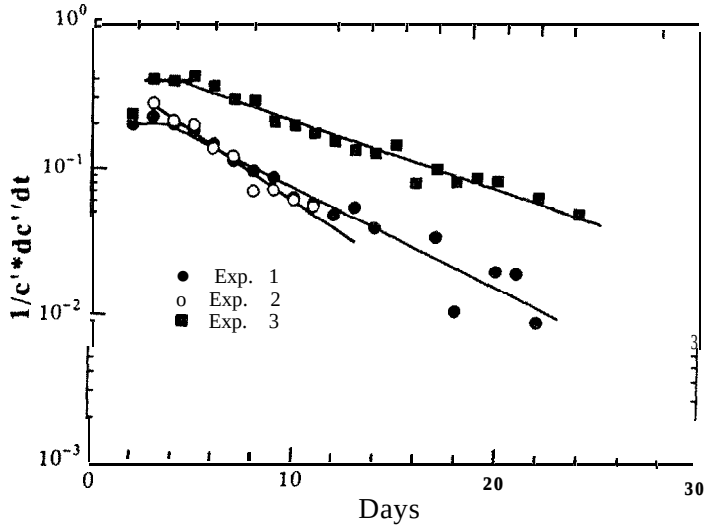

Fig. 9. Relationships of specific growth rate (ordinate) with the culture period (abscissaa, days).

Based on the regression lines obtained from experiment No.3 (Fig. 9), the cell density in the stable condition may be estimated to reach the level of 1300 x 104 cells/ml.

Our information indicates that the continuous measurement of total attenuation coefficient will give us, with the succeeding analysis of the growth curve, the attractive possibility of monitoring the duration of the stationary period and the cell number of the phytoplankton of Nannochloropsis species.

SUMMARY AND CONCLUSION

1. The present condition of continuous measuring systems for fish larval production in Japan is briefly reviewed from the viewpoint of their functions.
2. Current models of the monitoring system are effective for important parameters of water quality as well as for regulating the medium in phytoplankton culture.
3. These models are described in terms of their composition and peculiarities, with necessary mathematical background for monitoring sensors.
4. Advanced systems for the quantitative control of fish larval production will depend on an effective integration of numerical data available from the systems in question with those provided by empirical technologies for breeding management, through further development of electronic monitors.

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Reducing the Environmental Impact of High Density Fish Production: An Integrated Approach to Solids Treatment for Recirculating Aquaculture Systems Using Expandable Granular Biofilters

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ABSTRACT

Over the past decade, regulatory agencies have begun to view the environmental impact of wastes discharged from high density flow-through fish production systems witb increasing concern. At the same time, recirculating aquaculture systems have gained wider acceptance because of their ability to reduce waste discharges, improve quality control and reduce costs. The crucial processes that must be addressed in treating recirculating water are solids capture, biofiltration, aeration, degasification and ion balance. Designs that integrate two or more of these processes provide the greatest potential for cost reduction. The technology that is the focus of this paper is an expandable granular biofilter (EGB), which integrates solids capture and biofiltration in a single unit process, Backwash frequency is a major operational parameter of EGBs, influencing the volume of sludge produced and the nitrification rate. Computer and mass balance models are used to describe the relationship between solids residence time, sludge production and nitrification rates. The models show that infrequent backflushing decreases water loss and sludge production. although nitrification rates decline for extended solids residence times. Declining nitrification rates reflect decay of the accreting solids mass-which creates an internal ammonia and BOD load, decreasing the oxygen available to the nitrifiers because of heterotrophic competition for oxygen and impeded mass transfer as the bed becomes occluded. Nitrification appears to be optimized with solids residence time in the range of 2-3 days, for filters utilized as the primary solids capture device. The focus of this paper is: (1) primary in-filter solids stabilization; (2) the effect of in-filter solids stabilization on nittification; and (3) post-discharge. pre-disposal digestion of aquacultural solids.

INTRODUCTION

As waste discharges from flow-through systems have become the subject of increasing scrutiny by government regulatory agencies, recirculating systems are increasingly seen as a potentially effective means of minimizing the impact of large-scale aquaculture on water and environmental resources. High density recirculating aquaculture systems (RASs) allow for systematic optimization of costs and the environmental variables that determine the quality of the targeted aquatic species. Fish culturists in the United States are adopting RASs to enhance the production of highly valued products including tropical fish, ornamental goldfish and soft crabs. Researchers have been working toward integrating the functions and improving the efficiency of recirculating system components. The goal for recirculating systems research is extending the economic viability of RASs to commodity- priced products.

The five processes required to recondition water in a recirculating system are solids capture, biofiltration, aeration, degasification and ion balance. Solids capture is the

removal of feces, uneaten food and suspended bacteria, and it can be performed by settling tanks, microscreens or granular filters. Biofiltration is the conversion of dissolved organics and toxic nitrogenous compounds to bacterial biomass. Several common biofilters are rotating biological contactors (RBCs), trickling filters, fluidized beds and granular beds. Aeration is often provided by blowers and air stones, and it must be sufficient to meet the respiration demands of the culture species and the bacteria in the biofilter. Degasification is the removal of carbon dioxide that results from respiration in the system. Degasification can be achieved concomitantly with aeration by sparging a sufficient volume through air stones, or it can be performed separately in a packed column. Ion balance is necessary to correct potentially harmful chemical imbalances in systems where the water exchange rate is very low. Ion balance may be achieved by increasing the water exchange rate, addition of chemicals or denitrification for nitrate removal. Each of these crucial processes must be addressed to provide minimally acceptable water quality for the culture species.

System efficiency can be increased by the addition of optional processes: foam fractionation, ozonation, W disinfecting or denitrification. Foam fractionation removes organics that are not easily oxidized by bacteria, which helps control fine solids (<10 μ). A foam fractionator is simply a tube containing air stones, where fine particles form foam at the air-water interface for removal at the surface. Ozone is an effective oxidant used to remove refractory organic material, which contributes to color problems and bacteria. Ultraviolet (UV) light can be used to control many forms of bacteria algae and some viruses, reducing disease outbreaks. Denitrification addresses ion imbalance created by the nitrification process, removing excess nitrate and replenishing alkalinity. These processes, although usually considered optional, may become mandatory if stocking density is very high, the water reuse period is extended or endemic disease becomes a problem.

EXPANDABLE GRANULAR BIOFILTERS

Solids capture and biofiltration are often performed as sequential unit operations in RASs. For example, a settling basin followed by a rotating biological contactor is a widely-used configuration (Libey 1991, Van Gorder 1991). An expandable granular biofilter (EGB) performs both processes in a single operation. Early EGB configurations, such as the upflow sand filter (Burden 1988, Malone and Burden 1988), were limited in total ammonia nitrogen (TAN) conversion and solids capture by the fluidization characteristics of the sand These limitations were overcome in EGBs through the use of low-density floating plastic beads. Bead filters exhibit superior oxygen transport and are more easily cleaned than sand filters. Filtration of total suspended solids (TSS) is accomplished by settling, straining and interception within the granular bead matrix, and the bead bed operates simultaneously as a fixed film bioreactor. Periodic washing removes excess heterotrophic bacteria and highly organic solids, which accumulate in the interstitial spaces between the beads. Backwashing mitigates solids ammonification and occlusion of the filter bed, so it is the primary means of optimizing filter performance.

The benefit of this filtration approach stems from the extremely low water loss associated with solids removal, and the large specific surface area (1100 m^2/m^3) provided for the growth of bacteria. Low density polyethylene beads (3-5 mm in diameter) are employed as a filter media in an upflow pressurized configuration. The beads are less dense than water, float above the injection line and are retained in the filter by an overlying stainless steel screen. A propeller, embedded in the filter media, is activated for periodic cleaning. The filtration bed is underlain by a cone-shaped settling chamber, and as shown in Fig. 1, the filter has four operational modes.

During a typical filter operation (Step 1), nitrifiers—which convert toxic ammonia and nitrite to stable nitrate—and heterotrophic bacteria-which remove biochemical oxygen demand (BOD)—become attached to the filter media. Heterotrophic bacteria, which grow more rapidly, soon fill the pore spaces between the beads. As solids and bacterial biomass accumulate, solids ammonification increases and the transfer of oxygen and nutrients to the bacteria in the filter is impeded. Triggered by a timer, pressure sensor or computerized control unit, the propeller-driven backwash sequence is implemented, homogenizing the bed into the underlying settling chamber (Step 2). When the propellers are turned off, the beads float upward reforming the filtration bed, while the accumulated solids and the bulk of the heterotrophic bacteria become concentrated in the bottom of the settling chamber (Step 3). While a large part of the solids accumulating in the settling cone consist of bacterial biomass that are grown in the intervetting filtration cycle, a portion of the nitrifying and heterotrophic bacteria remain attached to the beads. After backwashing, the solids are allowed to compress, forming a concentrated sludge that can be removed with only minimal water loss (Step 4). Since water loss is negligible, the solids can be harvested frequently and little of the particulate BOD from solids excretion is expressed in the system. Thus, the bead filter is capable of mitigating extremely high wasteloadings.

Bead filters compare quite favorably to trickling filters and RBCs, which are clearly effective biofiltration units (Table 1). However, trickling filters and RBCs must maintain high porosity to avoid biofouling, which limits their specific surface and thereby their volumetric conversion

Table 1. A comparison of TAN conversion rates for common biofilter types.

Filter type	Areal TAN conversion (g/m^2-day)	Specific surface area (m^2/m^3)	Volumetric TAN conversion (g/m^3-day)	Reference
RBC	0.280	150	41	Mississippi Power and Light demonstration facility, Greenville, MI (Malone et *al.* 1993)
Upflow sand	0.064	2350	152	Commercial softcrayfish facility (Burden 1988)
Hydraulically washed EGB	0.23 1	1230	286	Experimental scale cattish system (Wimberly 1990)
Mechanically washed EGB	0.291	1050	308	Mississippi Power and Light demonstration facility, Greenville, MI (Malone et *al.* 1993)
Fluidized bed	0.284	2350	633	Experimental scale chemically fed (Thomasson 199 1)

capacity. On the other hand, fluidized beds display superior volumetric nitrification rates, but they must be used in conjunction with a solids capture device. Use of a bead filter for nitrification, in lieu of a fluidized bed, is predicated on the assumption that integrated treatment is more cost effective. That is, a bead filter sized for nitrification will be less costly than a properly sized solids capture device and a fluidized bed.

SLUDGE PRODUCTION

All the sludge generated from a recirculating aquacultural system can be equated to the feed. Assuming a typical feed conversion ratio of 1-2 kg feed/kg fish, and neglecting the impact of uneaten food, 80% of the feed (on a dry mass basis) put into an aquacultural system will eventually be wasted as fish excretion products (Hopkins and Manci 1989). Sludge volume is a major factor in designing a waste treatment system for effluents. Sludge volume generated from a recirculating system is controlled by the amount of solids produced (measured as kg of dry weight solids) and the degree to which the solids are concentrated in the effluent stream. Total sludge production from a recirculating system can be estimated by considering direct fish excretion, solids breakdown and biofloc production from soluble BOD excretion. The concentration is controlled by the solids removal technique employed to capture solids from the recycled stream. Solids production can be quantified through a mass balance that considers the major solids fluxes:

$$d(M_s)/dt = F*M_o(E_s + Eb*YH) - k_s*M_s*S_v - H_s*M_s \quad (1)$$

Where:

M_s is the mass of solids in the filter bed (mass);
M_O is the mass of cultured species (mass);
E_s is the solids excretion ratio (mass TSS/mass feed);
E_B is the dissolved BOD5 excretion ratio (mass BOD5/mass feed);
Y_H is the heterotrophic yield (mass VSS/mass BOD,);
k_s is the solids decay rate (d-l);
S_v is the volatile solids fraction (unitless); and

$$H_s = h_f f_b \quad (2)$$

Where: H_s is the solids harvest rate (d-l);
h_f is the solids harvest fraction (unitless); and
f_b is the backwash frequency (d-l).

The direct solids excretion ratio (E_s) has been observed as 0.40 (Speece 1973) to 0.52 kg/kg feed (Liao and Mayo 1974) for trout and 0.43 kg/kg feed for catfish (Wimberly 1990). Other reported TSS excretion rates for catfish ranged from 0.18 to 0.69 kg/kg feed (Page and Andrews 1974, Ruane *et* al. 1977). Solids excretion rates clearly vary with species, temperature and feeding rates. However, values of E_s in the range of 0.3 to 0.5 are common.

The soluble five-day biochemical oxygen demand (BOD,) excretion rate can also be expressed as a fraction of the feeding rate. Based upon a study of channel catfish, Murphy and Lipper (1970) reported the soluble BOD_5 as 58% of the total BOD, excreted, whereas, BOD_5 in particulate matter was 42%. Wimberly (1990) found that the soluble BOD_5 excretion ratio was 0.05 kg BOD_5/kg feed, or about 23% of the total BOD, excreted.

The first-order solids decay rate (k_s) at 20°C varies with solids residence time (SRT); k reportedly varies from $0.124\ SRT^{-0.594}$ (Z. Ning, Louisiana State University, Baton

Rouge, pers. commun. 1995) to 0.278 $SRT^{-0.518}$ (L. Wang, Louisiana State University, Baton Rouge, pers commun. 1995). The volatile suspended solids (VSS) fraction of catfish excretions has been reported as about 0.9 by Wimberly (1990) and as about 0.7 for Kemp' s ridley sea turtles (Malone et al. 1990), red swamp crawfish (K.M.Cange, Louisiana State University, Baton Rouge, pers. comm. 1987) and blue crabs (Burden 1988).

The solid production from biofiltration depends on the growth of bacterial biomass during the breakdown of dissolved organics (BOD_5) and nitrification. Considering ammonia nitrogen excretion rates of 1.8 to 4.6% of the feeding rate (Page and Andrews 1974, Ruane *et al.* 1977, Wnnberly 1990) and the stoichiometry of nitrification cited by Wheaton (1977). biomass production due to nitrification is negligible at 0.3% to 0.9% of the feeding rate. The biomass production due to dissoved BOD, consumption, on the other hand, is more significant. For example, if the soluble BOD, excretion is 0.05 kg BOD_5/kg feed, as reported by Wimberly (1990). biofloc production will be about 6% of the feed rate.

H_s is related to the solids residence time (SRT) by:

$$SRT = 1/H_s \quad (3)$$

The solids harvest rate for a given bead filter can be determined by washing the filter repeatedly and estimating the harvest fraction, and SRT can then be estimated for a variety of backwashing sequences. With a backwash frequency of once every two days, a filter with a harvest fraction of 0.5 will have a H_s of 0.25 and an SRT of 4 days.

The sludge production constant Sp (kg/day) from the system is defined as:

$$S_p = H_s * M_s \quad (4)$$

The concentration of the sludge stream (S_c, kg/m^3) is determined by the efficiency of the sludge separation process and the amount of flushing or washdown water (Q_s, m^3/day) required for the sludge removal.

$$S_c = S_p/Q_s \quad (5)$$

Calibration of a computer model, using Equations 1 through 5, resulted in k_s = 0.665 d^{-1} for an SRT of 3.5 days. Because aquaculture sludge is partially digested in the filter, k_s values for sludge with a high SRT are usually lower than those observed for municipal waste (0.48 $SRT^{-0.415}$, Rich 1982).

Equations 1 through 5 can be used to estimate sludge production from a proposed recirculating configuration. Table 2 illustrates that the mass of total Kjeldahl nitrogen and the volume of sludge generated by fish is comparable to that of other commercially raised animals.

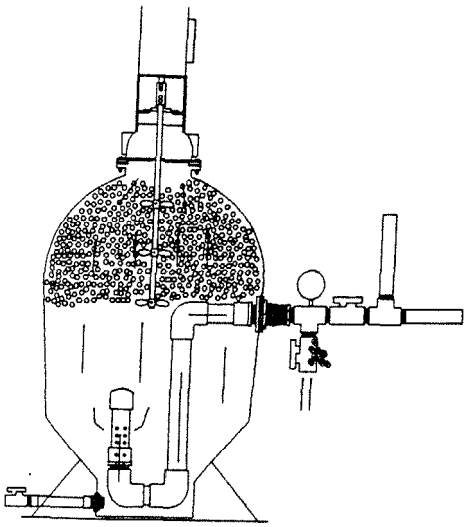
DISCUSSION

Direct discharge of untreated aquacultural solids to receiving streams, e.g., in flow-thmugh systems, can cause a variety of problems including oxygen depletion, nutrient enrichment, loss of water clarity and destruction of benthic communities by the formation of sludge deposits. However, in recirculating systems, water discharge rates ar enegligible, e.g., 5-10%/day, and effluent streams can be clarified, separating and concentrating settleable solids prior to discharge. Although the sludge itself must be disposed of throug hland application or landfilling, the material has been partially oxidized by biofilter bacteria. The two issues most important to successful aquacultural solids management are (1) managing primary in-filter solids stabilization to minimize its impact on other critical system functions and (2) selecting the most appropriate post-discharge treatment option.

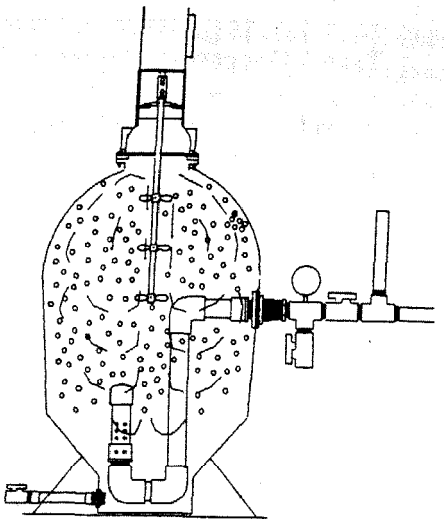
As can be seen from Equation 4, the mass of sludge produced from a recirculating system (S_p) is the product of the solids content of the system (M,) and the sludge harvest rate (H,). However, Hs and SRT are inversely related through Equation 3. Decreasing H_s tends to decrease sludge production as the amount of solids decay increases with SRT, but solids ammonification and mass-transfer constraints also increase with SRT, causing apparent nitrification to decline. Chen et al. (1993) applied Equations 1 through 3 to a model of a hypothetical finfish system of a 1000 kg capacity. Utilizing constant values for E_s=0.4, E_B=0.05, Y_H=0.4 and k_S=0.36, a reduction of about 50% in discharged sludge mass was achievable by manipulating the bead filter backwash frequency. However, the corresponding increase in sludge mass held in the system ultimately increases aeration and degasification burdens on the recirculating system and causes the apparent nitrification rate to decline. This phenomenon constrains efforts to manipulate SRT for purposes of sludge volume reduction. Naturally, the SRT-nitrification relationship will define the maximum sludge reduction and the backwash frequency should be manipulated to optimize nitrification.

Sludge management policies should focus on increasing S_c (Equation 5), since the concentration of solids varies dramatically with the type and management of the solids control device employed in the recirculating loop (Table 3). Additionally, the sizing criteria and cost of the stabilization and disposal options depend upon the volume of sludge produced, If the solids capture device in the production system is not capable of concentrating the solids, then an external clarifier should be used to achieve the desired sludge density. Consideration should be given to partitioning sludge stabilization between internal and post-discharge treatment processes. Integrated design allows for overall minimization of treatment costs, reduction of the potential for adverse environmental impact and enhanced RASE efficiency.

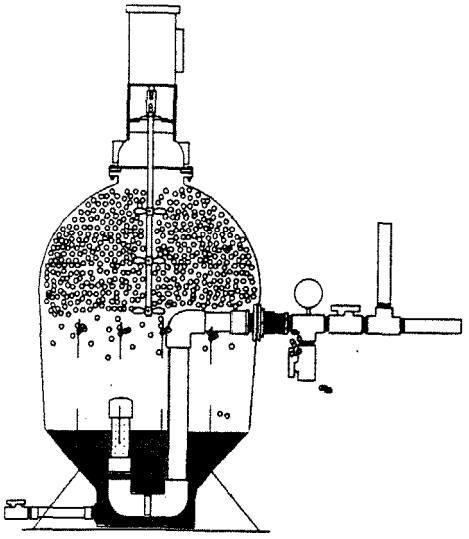
Bead filters are designed with internal settling cones, to facilitate single stage sludge concentration (Fig. 1). Additionally, the sludge retention time can be controlled



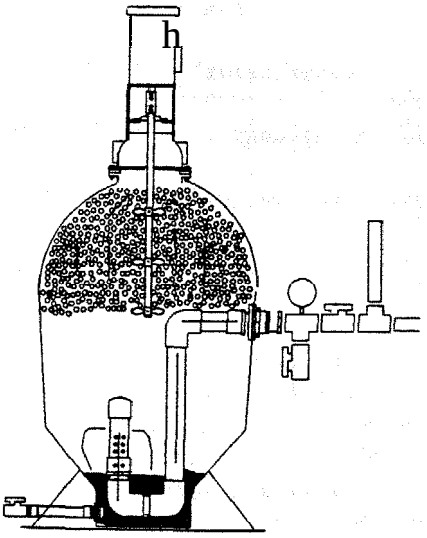
Step 1: Filtration



Step 2: Backwashing



Step3: Settling



Step 4: Solids Removal

Fig. 1. The four operational modes of an expandable granular biofilter (EGB).

Table 2. Comparison of waste generation rates of commercial animals (kg/d).

Animal	BOD	TSS	TKN	Sludge vol.	Reference
Fish	1.13	3.9-6.3	0.2-2.32	65-630	Chen *et al. (* 1993)
Beef cattle	1.6	9.5	0.32	30	Middlebrooks *et al.* (1982), Overcash et *al.* 1983)
Dairy cows	1.4	7.9	0.51	51	"
Poultry	3.4	14	0.74	37	"
Swine	3.1	8.9	0.51	76	"

Table 3. A **comparison of slude TSS concentrations from several sources.**

Source	Sludge TSS concentration	Reference
Upflow sand filter	0.005-0.015%	Malone and Burden (1988)
Sand filtration	0.01-0.02%	Metcalf and Eddy (1979)
EGB	0.05-0.5%	Chen et *al.* (1993)
Primary sedimentation	1-6%	Chen *et al.* (1993)

Table 4. A **comparison of sludge stabilization options.**

Method	Advantages	Disadvantages
Anaerobic lagoon	High organic loading capacity Low maintenance	Odor
Aerated lagoon	High organic loading capacity Low area requirement	Large energy consumption Moderate maintenance
Composting	Useful end-product	Dewatering required Moderate capital expense
Anaerobic digester	High organic loading capacity Methane generation	Complicated management High maintenance

by the frequency, duration and vigor of the backwash. Higher sludge retention times (2-5 days) tend to encourage the biodegradation of solids concomitant with enhanced nitriftcation rates and decreased water losses. Increasing the settling time after a backwash is another means of significantly increasing sludge density. These factors are important to the linkage of internal and external sludge treatment processes.

High nitrogen contents (4-6%), phosphorus levels of about 2% and the absence of contaminants, such as heavy metals, make aquacultural sludge attractive as a fertilizer (Willett and Jakobsen 1986). Direct land application has proven feasible in areas with dry climates where the high moisture content of the sludge is considered beneficial. In wet climates, additional stabilization of sludge may be required to avoid odor and runoff problems. Table 4 lists some of the more common methods for digesting sludge, along with the important advantages and disadvantages of each method.

Anaerobic lagoons are inexpensive and easy to operate. However, because of their odor, they are seldom suited for any but the most remote locations. Aerated lagoons have an organic loading capacity similar to anaerobic lagoons with no offensive odor, but aeration equipment is initially expensive with continuing operations and maintenance costs. Composting yields soil conditioner, which is a commercially valuable end-product. However, composting reuqires mechanical dewatering prior to staking in static piles or windrows, and static piles must be aerated while windrows require periodic mixing. Anaerobic digesters are very popular for stabilizing sludge from municipal waste treatment plants, but they are expensive and their management requires specialized knowledge of their microbiology.

SUMMARY

Recirculating aquaculture systems provide a means for

actively controlling the quality of the aquatic species being produced. RASs also mitigate the negative environmental impact caused by the continuous discharge of organic and mineral contaminants by large-scale aquaculture production systems. An integrated approach to solids treatment utilizes the synergy between internal and external processes to reduce costs. Manipulation of the bead-filter backwash regime can result in a substantial reduction in discharged sludge mass, through biodegradation, without adversely affecting nitrification. Dilute sludge produced by backwashing or washdown operations can be concentrated by internal settline or by external clarification processes prior to stabilization and disposal. Aerobic and anaerobic processes with extensive track records are available to reduce the easily biodegradable portion of discharged sludge, minimizing the volume of sludge for final disposal. As a final disposal option, land application appears most feasible for rural areas, whereas landfilling may be most appropriate for urban areas.

In this paper, we have focused on solids discharge because it presents the greatest threat of environmental degradation, particularly oxygen depletion and destruction of benthic communities. However, the discharge of nutrients, i.e., nitrate and phosphorous, can cause eutrophication of the receiving water. The major problem associated with nutrient enrichment is an algal bloom, providing the basis for an oxygen crash when water quality or environmental conditions change. Nitrate removal can be accomplished by denitrification within or external to the RAS. If denitrification is provided within the RAS, some of the alkalinity lost in the nitrification process will be replenished. Phosphorous removal methods are expensive and the reformulating of aquatic feeds to increase metabolically available phosphorous is the most promising means of phosphate discharge reduction.

ACKNOWLEDGMENTS

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Management of a Seawater Recirculation Fish Culture System for Japanese Flounder

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ABSTRACT

Rearing experiments of the Japanese flounder, *Paralichthys olivaceus*, were carried out under high fish density conditions using a closed seawater recirculation system of 22 m^3 in total water volume. After 330 days of rearing, fish grew to 480 g in mean body weight (initial: 3.5 g) and total fish biomass in the system was 844 kg. Rearing density per unit volume of water in the system reached 38.4 kg/m^3. Because 6 and 9 m^3 of the rearing water exchanged with fresh seawater at the measurement of fish body weight, the product per unit volume of seawater used was 22.6 kg/m^3. Ammonia and nitrite concentrations of the rearing water were maintained under 1 mg-N/L during the first 100 days; however, after that, ammonia concentrations fluctuated between 0.5 and 7.8 mg-N/L and nitrite between 1.0 and 4.0 mg-N/L. Nitrate concentrations increased with the cumulative amount of feed and reached 359 mg-N/L on the 190th day. Then they decreased from 312 to 120 mg-N/L. during the period from the 220th to the 263rd day, because of the occurrence of denitrification resulting from the formation of local anaerobic areas in the system. Although there were some fluctuations of ammonia, nitrite and nitrate concentrations, these results indicate that intensive culture of Japanese flounder is possible with a small quantity of seawater without daily water exchange and without direct impact on the aquatic environment by using the closed seawater recirculation system.

INTRODUCTION

Most of the saltwater fish culture is managed with cages on the coast of Japan. Some kinds of fish, such as Japanese flounder, are reared in tanks built on land with flowing water pumped from the sea. This fish culture method is called the flow-through method. Because these two types of fish culture depend on the natural sea and seawater, fish growth and management of the systems are affected by seasonal changes, weather conditions and other factors. Moreover, these two open fish culture systems discharge feces of rearing fish and leftovers directly into the sea. Therefore in some areas, they cause deterioration of the culture ground and water pollution (Piedrahita 1994, Van Rijn 1996).

On the other hand, closed water recirculation fish culture systems have the advantage of using very small quantities of water for fish production compared with flow-through systems. Therefore, it is easier to maintain an optimum temperature for rearing species in the closed Systems. Closed systems have another advantage of less direct impact on the aquatic environment than open systems when waste materials from the systems are managed prop erly.

Since 1986, we have been studying closed seawater recirculation systems for Japanese flounder from the viewpoint of obtaining optimum conditions using electric power and also reducing the impact on the aquatic environment, Honda et *al.* (1991) reported on the possibilities of intensive culture of Japanese flounder without the wasteful use of seawater, with a closed seawater recirculation system. The present report deals with a rearing experiment of Japanese flounder using a large closed seawater recirculation system of 22 m^3 in total water volume.

MATERIALS AND METHODS

RECIRCULATION SYSTEM AND ITS OPERATION

The system was planned to produce 2,000 fish of 500 g in body weight, which is the minimum commercial size for cultured flounder in Japan. The system consisted of a fish tank, a settling tank, two biological filters, a heating-cooling unit, a circulation pump, a UV light unit and three air-blowers. The minimum necessities of filter media, aerator capacity, seawater for operation and bottom area of fish tank were calculated based on the results of other experiments, such as upper rearing density, respiration rate, ammonia excretion rate of fish and ammonia oxidatian rate of biological filters (Honda 1988, Honda *et al.* 1991, Kikuchi *et al.* 1990, 1991, 1992, 1994) as shown in Fig. 1. The schematic diagram of the system is shown in Fig, 2.

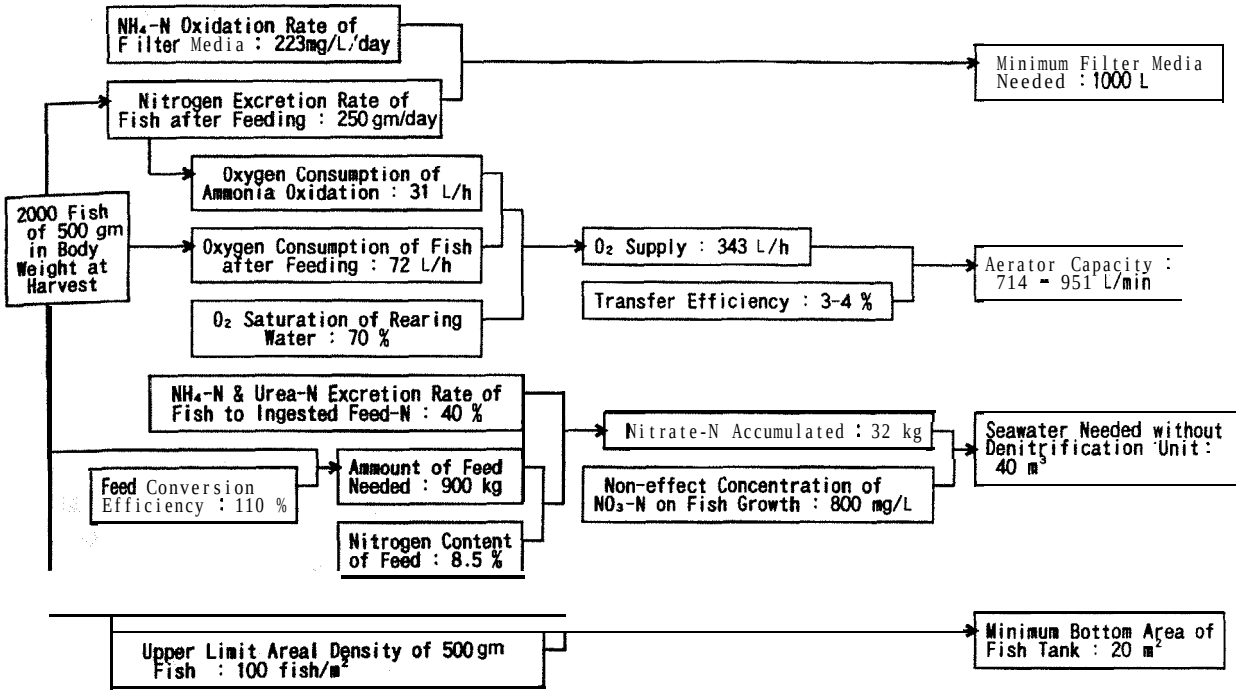

Fig. 1. Outline of design process of closed systems for Japanese flounder.

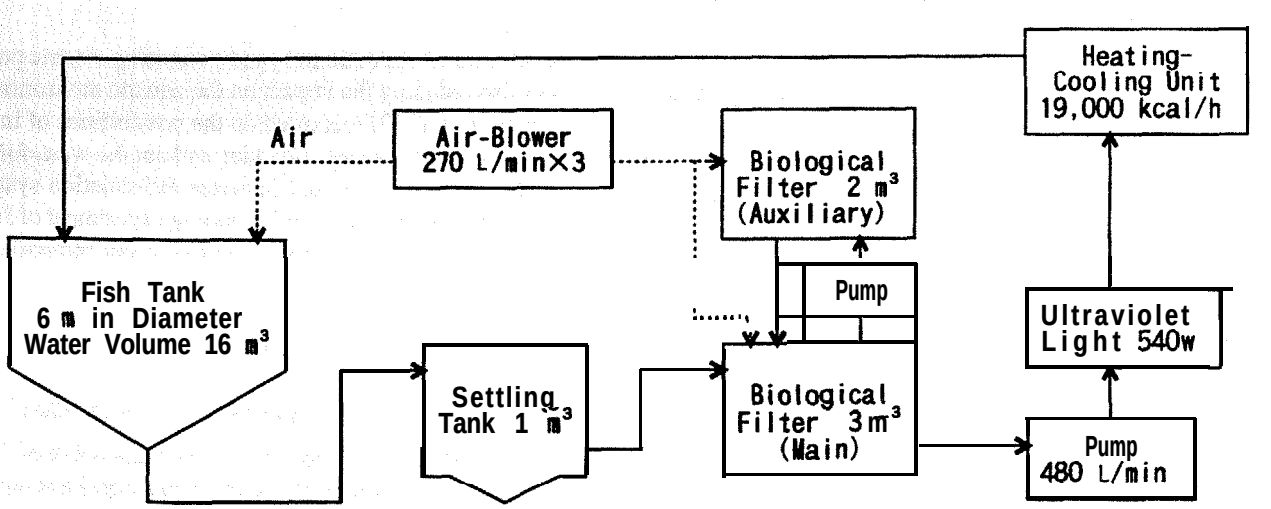

Fig. 2. Schematic diagram of the closed seawater recirculation system.

The fish tank was 6 m in diameter and the bottom sloped to 0.1 m in the center. Average water depth was 0.6 m and the water flow rate at the inlet was adjusted to 0.4 m^3/min. The biological filters consisted of a 3-m^3 main filter tank and a 2-m^3 auxiliary filter tank connected in bypass with a pump of 0.1 m^3/min. Filter media of 1.3 m^3 of small net-type plastic, which have a specific area of ca. 350 m^2/m^3 and were previously well conditioned, were placed in the main filter tank, and 0.5 m^3 of filter media were also placed in the auxiliary tank. A submersible water pump with a capacity of 0.1 m^3/min was placed in the main biological filter tank on the 265th day to circulate water in the tank sufficiently. The settling tank of 1 m^3 was equipped with the system just before the main biological filter tank. To determine the production rate of sediment in the settling tank and biological filters, sediment was not removed from the tanks during the first 115 days. After that, sediment in the tanks was removed every two months. Natural seawater used in this experiment was collected at the coast near Onjuku, Chiba Prefecture. Because the optimum temperature for the growth of the flounder is 20 to 25°C (Iwata ef al. 1994), water temperature was controlled at 25°C in summer and 20°C in winter. The pH of the rearing water was maintained between 7.0 to 7.5 with $NaHCO_3$. To adjust the salinity of the rearing water, fresh well water was added corresponding to the evaporated volume. Dissolved oxygen of the rearing water in the system was maintained with air-blowers and air-stone diffusers placed in the fish tank and the biological filters. The numbers of blowers were increased from one to three with growth of the fish. The total water volume in the system was adjusted to 22 m^3. Six and 9 m^3 of rearing water were exchanged with fresh seawater at the time of measurement of fish body weight on the 193rd and 242nd days.

REARING OF FISH

On April 21, 1994, 2000 fish of 3.5 g in mean body weight, obtained from a hatchery in Mie Prefecture, were stocked in the fish tank at a density of 70 fish/m^2. The fish had been starved for 72 h before the measurement of body weight. The fish were fed until satiation on a commercial diet containing ca. 8.5% of nitrogen twice a day from Monday to Friday and once on Saturday and Sunday. The feedings totaled 313. The rearing continued until fish reached ca. 500 g in body weight. To estimate the increase of fish biomass, body weights of 50 to 150 fish were measured at ca. 30- to 40-day intervals. On the 242nd day and at the end of the experiment, body weights of all fish in the system were measured. The numbers and body weights of dead and removed fish were recorded to correct the nitrogen budget in the system.

ANALYSIS OF REARING WATER

Ammonia concentration was determined by the indophenol method. Nitrite concentration was determined by the Griess-Romijn method. Nitrate concentration was measured with an ion chromatographic analyzer (IC-500, Yokogawa Electric Co., Tokyo). Oxygen concentration was measured with a DO meter (YSI model 58, Yellow Spring Instruments Co. Inc., Yellow Spring). The pH was measured with a pH meter (M-8, Horiba Seisakusho Co., Kyoto). These concentrations were measured routinely twice a week, usually on Tuesday and Friday. Water for analysis was sampled in the morning before feeding.

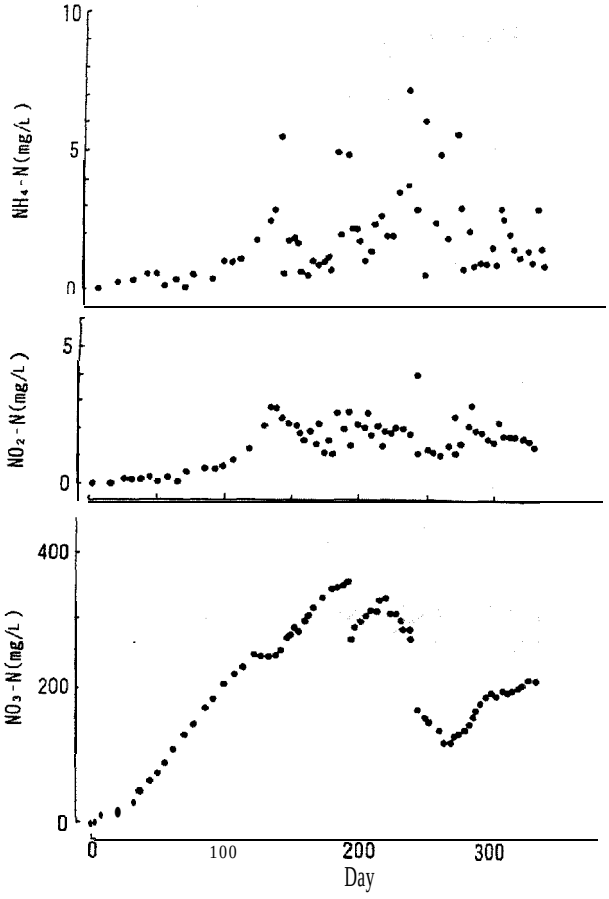

Fig. 3. Fluctuations of ammonia, nitrite and nitrate concentrations in the rearing water of Japanese founder.

RESULTS AND DISCUSSION

WATER QUALITY

Ammonia and nitrite concentrations of the rearing water were maintained under 1 mg-N/L during the first 100 days; however, after that, ammonia concentrations fluctuated between 0.5 to 7.8 mg-N/L and nitrite between 1.0 to 4.0 mg-N/L (Fig. 3). The mean concentrations of ammoma and nitrite were 1.95 ± 1.62 and 1.62 ± 0.88 mg-N/L (mean ± SD), respectively. As shown in Fig. 3, nitrate concentrations increased with the cumulative amount of feed (see Fig. 7) and reached a maximum of 359 mg-N/L on

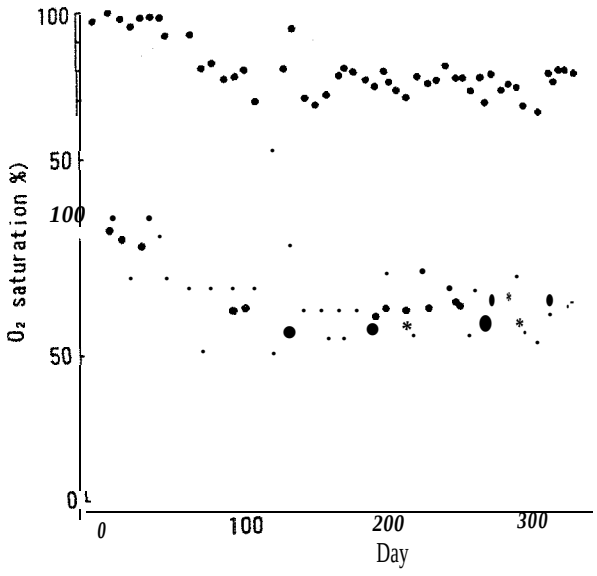

Fig. 4. Dissolved oxygen levels of rearing water in the fish tank (upper) and at the outlet of the main biologicaljilter (lower).

the 190th day. After **exchange** of 6 m^3 of water, nitrate concentration decreased to 277 mg-N/L on the 193rd day and then increased until 312 mg-N/L. Nitrate concentrations decreased to 120 mg-N/L during the period from the 220th to the 263rd day, because of the occurrence of denitrification resulting from the formation of local anaerobic areas in the system and the water exchange of 9m^3 on the 242nd day. After the placement of a submersible water pump in the main filter tank on the 265th day, nitrate concentrations increased and reached 2 13 mg-N/L, and fluctuations in ammonia concentrations became smaller. From these facts, it was concluded that fluctuations of ammonia, nitrite and nitrate concentrations resulted from insufficient circulation of water in the biological filter tank.

Dissolved oxygen levels in the rearing water in the fish tank were more than 70% of saturation through most of the experimental period. After the 100th day of rearing, levels of dissolved oxygen measured at the outlet of the main biological filter often decreased to 50% as shown in Fig. 4. From these facts, it is possible that some local anaerobic areas less than 30% in saturation levels were formed in the biological filter and brought about denitrification.

The total amount of $NaHCO_3$ added to the rearing water for pH adjustment was 65 kg. The total volume of well

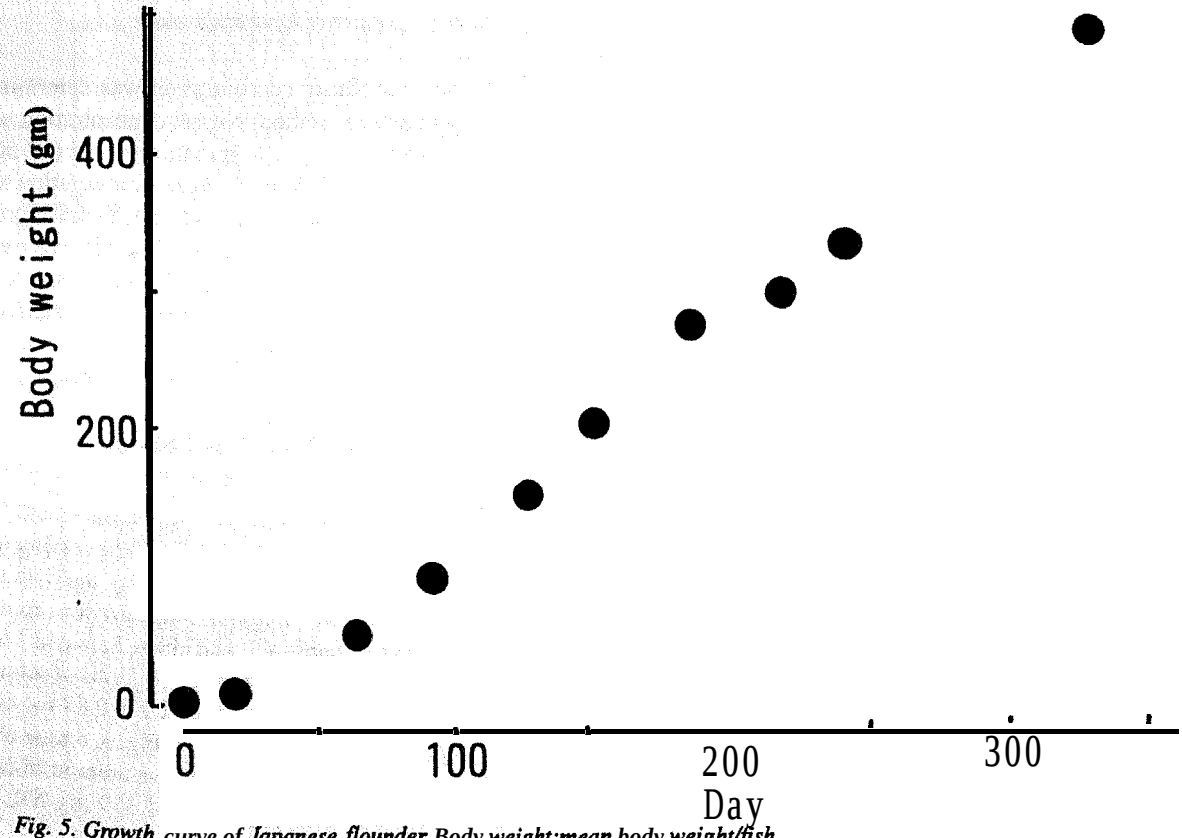

Fig. 5. Growth curve of Japanese flounder. Body weight:mean body weight/fish.

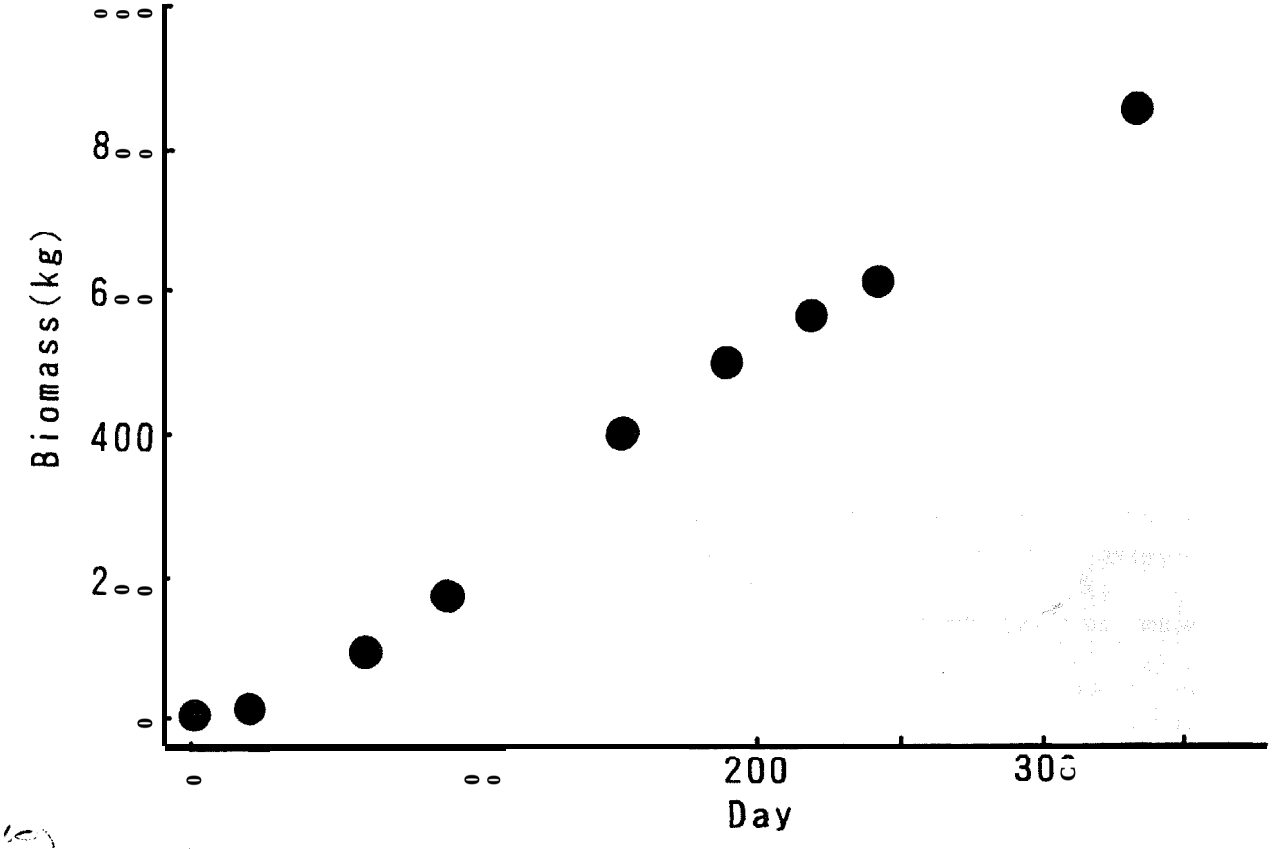

Fig. 6. Increase of biomass in the fish tank.

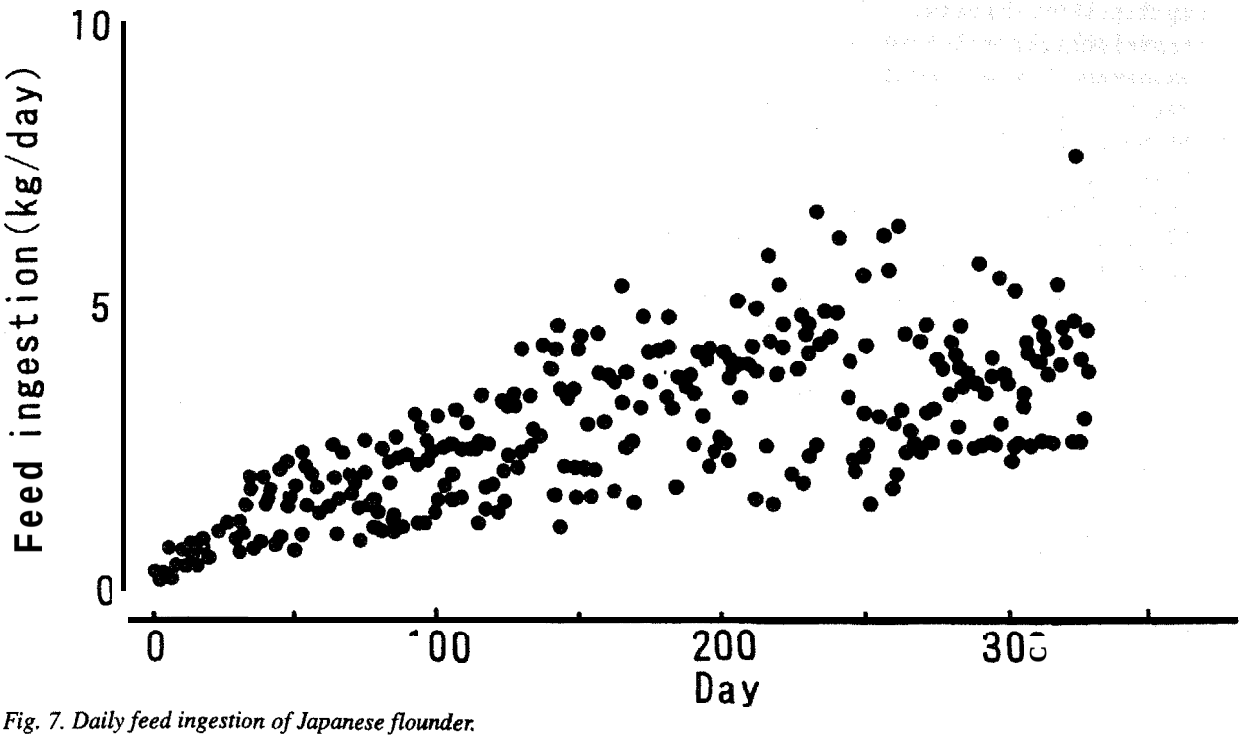

Fig. 7. Daily feed ingestion of Japanese flounder.

Table 1. Results of 330 days rearing of Japanese flounder.

Survival rate(%)	88.0
Final biomass(kg)	844.3
Weight increment(kg)	837.3
Feed ingested(kg)	842.0
Feed conversion efficiency(%)[a]	99.5
Density per unit volume of water(kg/m^3)	38.4
Density per unit area of fish tank(kg/m^2)	30.1
Production per amount of water used(kg/m3)	22.6

[a] *FCE: Fish in wet/ feed in almost dry*

water used to adjust the salinity was 10.3 m^3. Concentrations of suspended solids in the rearing water measured occasionally during the operation were determined to be almost 3 to 5 mg/L, although the concentrations increased from 10 to 11 mg/L just after feeding.

FISH GROWTH

Although high concentrations of ammonia and nitrite were sometimes observed, the fish grew normally compared with our past experimental results and reached 480 g in mean body weight after 330 days rearing, as shown in Fig, 5. The total fish biomass in the fish tank reached 844.3 kg (Pig. 6). As shown in Pig, 7. daily feed ingestion increased with fish growth and a maximum of 7.7 kg/day was observed on the 306th day. It was estimated that ca. 260 g of ammonia was excreted. The survival rate was 88% and the total weight increment was 837.3 kg. Total feed weight ingested by fish was 842 kg. so feed conversion efficiency (fish: wet/feed: almost dry) became 99.5%. The corrected feed conversion efficiency added to the weight of dead and removed fish was ca 110%. The figures of feed conversion efficiencies were almost equal to our past experimental results and the efficiencies of juvenile Japanese flounder reared by flow-through systems (Saitoh *et al.* 1990). The fish density per unit volume of water in the system reached 38.4 kg/m^3 and areal density in the fish tank was 30.1 kg/m^2. Since the weight increment was 837.3 kg and the total amount of seawater used for rearing was 37 m^3, the product per unit volume of seawater used reached 22.6 kg/m^3 (Table 1). This result implies that 1 kg of flounder was produced in only 44 L of seawater

NITROGEN BUDGET

Nitrogen budget in the system was estimated based on the total feed weight ingested, the weight increment and the total weight of dead and removed fish from the system. In this estimation, the nitrogen content of feed was 8.5% and fish was 3.7% based on our analyses. As shown in Fig. 8, about half (33.8 kg, 47.3%) of the nitrogen contained in the feed (71.5 kg, 100%) ingested by fish was transferred into fish protein, and another half (37.7 kg, 52.7%) was excreted from the fish into the rearing water. This budget agreed with an earlier determination of nitrogen excretion rate of the flounder (Kikuchi *et* al. 1991), although the proportion of excreted nitrogen was slightly higher in this study. On the basis of the nitrogen excretion rate of the flounder, excreted ammonia, urea and feces were estimated at 27.5 kg (38.5%), 3.8 kg (5.3%) and 6.4 kg (8.9%), respectively. Nitrate accumulated in the rearing water was estimated at 31.3 kg (43.8%) when all of the ammonia and urea excreted from the fish were oxidized to nitrate. About 70% (21.9 kg) of the nitrate accumulated was removed from the rearing water by denitrification. Feces nitrogen was not traced completely in this study. The sediment in the settling tank and the biological filter tanks was collected on the 115th day. The total weight of the sediment was 5.2 kg dry weight. This weight was ca. 1/7 of the estimated feces weight based on total feed weight ingested during the first 115 days and the nitrogen excretion rate of the flounder. Possibly a substantial amount of organic matter in the feces was decomposed and the inorganic nitrogen was released into the rearing water. In the settling tank, a large number of polychaetes (*Capitella* sp.) appeared around the 200th day of operation. The results of our preliminary experiments showed that an individual polychaete ingested 0.7 mg (dry weight) of the feces of Japanese flounder (33.7 mg-N/gm) and excreted 0.27 mg of feces (25.4 mg-N/gm)/day at 25°C (Fig. 9). In this process, ca 70% of the nitrogen included in the feces of the flounder was reduced. From these results, it is considered that a large part of the nitrogen in the feces of the flounder was transferred to the polychaete during the last 130 days.

OPERATION COST

In the 330 days of operation, 2000 seedling fish, 842 kg of feed and 41,237 kWh of electricity were used to produce ca. 840 kg of flounder. Unit prices of these items were 120 Japanese yen/fish, 350 yen/kg of feed and 11 yen/kWh, respectively. Therefore, the cost for 1 kg production of the flounder was 1180 yen (ca US $11). This is not a great difference from the total cost of seedling, feed and electricity in the current flounder culture with open flow-through systems, notwithstanding the operation of a heating-cooling unit to keep the optimum temperature for growth of the flounder. The cost of the closed sea-water recirculation system used in this study was 1.5 to 2 times higher, however, compared with the flow-through systems consisting of 6-m diameter fish tanks and other systems.

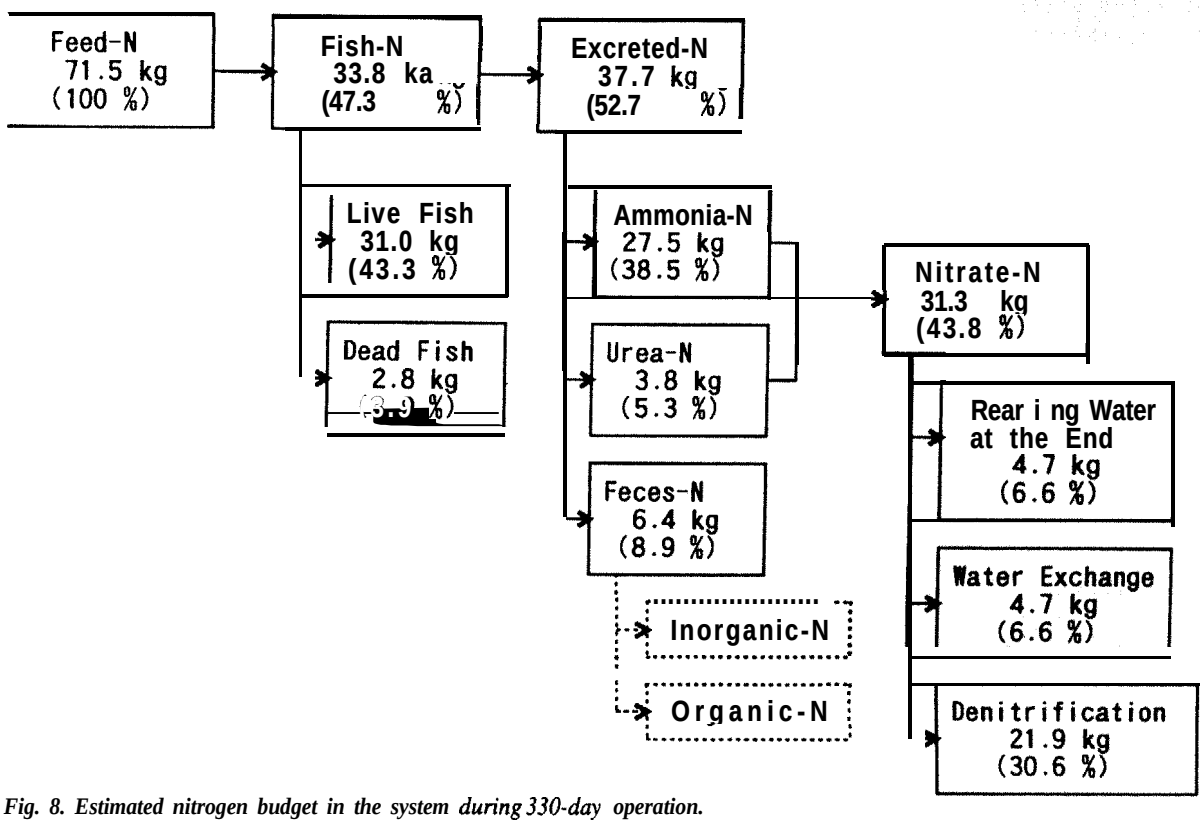

Fig. 8. Estimated nitrogen budget in the system during 330-day operation.

CONCLUSION

The results of this rearing experiment demonstrate that intensive culture of Japanese flounder is possible with a small quantity of seawater, without daily water exchange and without direct impacts on the aquatic environment by using a large closed seawater recirculation system, although there is some need of an economic feasibility study on the commercial operation of the system.

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