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cc: Roger Giegelhaus, Thurston County
    Diane Cooper, Taylor Resources

Re: Pacific Shellfish Institute (PSI) Aquaculture Initiative:
Ecological Characteristics and Carrying Capacity of Suspended Shellfish Culture Systems

Dear Independent Technical Reviewers:

At the request of Roger Giegelhaus, Thurston County Development Services, and Diane Cooper, Taylor Resources, I am sending each of you a copy of the PSI project description for research to begin this fall. Using established mussel culture operations in Totten Inlet and Penn Cove as the study sites, PSI proposes to quantify the interactions of bivalves cultured in intensive raft systems with the surrounding environment by examining linkages between the oceanographic and physiological processes and relationships with nutrient loading and ecosystem function.

The results of the PSI study will be a source of information to be used in the Taylor Resources’ North Totten Inlet Mussel Culture EIS. For this reason, the County and Taylor Resources are interested in your review and comment on the proposed research plan and methods. We would like to discuss the PSI research project and other matters related to the EIS (including the status of the contract for your services) in a conference call as soon as a date and time can be identified that works for everyone. Dates currently under consideration include Wednesday morning, August 29th, or Friday afternoon, September 7th. A time will be established as soon as the date is confirmed. Please review the enclosed document by the end of the day Tuesday, August 28th, in the event that a conference call can be arranged Wednesday morning. Final confirmation of the date and time of the conference call will occur via e-mail.

Several other aquatic environment studies are proposed by Kenn Brooks, Ph.D., Aquatic Environmental Sciences, to respond to requirements identified by the Thurston County Hearings Examiner. The scope of work and protocols for these studies will be available soon for your review.

Sincerely,

Vicki Morris
AQUACULTURE INITIATIVE PROJECT DESCRIPTION

Ecological Characteristics and Carrying Capacity of Suspended Shellfish Culture Systems

Introduction

Aquaculture and marine dependent animal and plant resource development face increasing obstacles to economic expansion nationally as a result of both real and perceived environmental impacts of these activities on the marine environment. At the same time, the economic value of fresh and saltwater aquaculture products provided to the national economy is considerable (estimated 1996 value $736 M), and increasing annually. With respect to marine bivalve aquaculture, extensive estuarine areas are currently used for commercial cultivation of shellfish. The production of bivalves on the U.S. west coast (California, Oregon, Washington and Alaska) is largely comprised of oysters and clams, which are cultivated using both bottom-based and suspended culture techniques (Chew, 1990; 1997), and mussels, which are cultivated almost exclusively using suspended (raft or floating long-line) systems (Figures 1, 2 and 3). With an annual production of approximately 3 million pounds live weight in Washington State alone, suspended mussel and oyster culture is predicted to increase significantly in coming years as U.S. and foreign demand for shellfish grows (Chew, 1996; G. King, personal communication). Oyster farmers in Alaska rely almost totally on suspended culture systems (Figure 3). These methods are also seeing practical application for the cultivation of scallops in northwestern Washington state, and pearl oysters in Hawaii and the western Pacific.

![Figure 1. Locations of representative suspended shellfish farms in Alaska and Washington. Top: Kenai Peninsula, Alaska, oyster farm sites; Bottom: northwest Washington State, locations of mussel, oyster and scallop farms.](image-url)
Figure 2. Suspended mussel farm in south Puget Sound. South Sound experimental site is at the circled location. A. Overall view of south Puget Sound (shaded); B. Totten Inlet; and C. Raft culture unit in Totten Inlet.
Shellfish aquaculture is increasingly recognized as an activity that has the capacity to alter natural marine systems or otherwise contribute to the co-opting of marine resources. Recent studies in France indicate that the flux of materials and the removal of seston from the water column by suspension-feeding oysters under intensive cultivation can have significant environmental impacts (summarized by Heral, 1998). This has great implications in the U.S. where intensive mussel and oyster culture is a relatively recent phenomenon and is subject to increasing oversight under the Endangered Species Act (ESA), Essential Fish Habitat (EFH) and Coastal Zone Management guidelines.

The goal of this study is to quantify the interactions of bivalves cultured in an intensive raft culture system to the surrounding environment by examining linkages between the oceanographic and physiological processes and relationships with nutrient loading and ecosystem function. Using the analytical methods previously developed it will not only be possible to gain a better understanding of these processes as they exist in the Pacific Northwest but it will also be possible to compare these conditions and processes to other systems around the world.

![Figure 3. Long-line suspended oyster farm and hanging basket trays (inset) in Alaska. Similar systems are in place in Washington and Oregon.](image)

**Current State of Knowledge**

It has been long recognized that dense assemblages of naturally occurring suspension feeding bivalves have the capability of significantly impacting nutrient cycling in shallow marine embayments (Boynton, et al., 1980; Dame, et al., 1980). A number of recent studies and literature reviews have demonstrated the significant role that suspension feeding bivalves can have in regulating phytoplankton abundance, benthic pelagic coupling, water clarity and annual nutrient cycling regimes via the removal of seston from the water column and via the production of biodeposits (Dame, 1996; Bayne and Warwick, 1998; Newell and Ott, 1999, Brooks, 2000). Most important have been the recent testing of models which help predict the biomass of mussels which can be cultivated in a specific location based mainly on measurements of seston flux and changes in bivalve biomass (e.g. Newell et al., 1998, Scholten and Smaal, 1998 and Grant and Bacher 1998). The predictive value of such modeling efforts is of particular importance to the future siting of shellfish aquaculture facilities, mussel or otherwise. Shellfish culture practices have recently come under significantly increased environmental and public scrutiny as natural resource agencies and government officials direct more attention towards protecting estuarine ecosystems for their biological productivity, complex habitats, and diverse assemblages of aquatic species. In addition, during recent years, the aquaculture industry has significantly increased the biomass of cultured bivalves within existing leases or shellfish beds as new species have come under cultivation and closures due to pollution have limited or restricted the spatial expansion in many locales. As a result, the density of cultured bivalves on existing beds and in suspended culture the water column has increased in some west coast estuaries.
Resource agencies, particularly those engaged in water quality enforcement and regulation, have traditionally viewed the commercial shellfish industry as a key indicator of a healthy estuarine environment. However, objective criteria are needed for shellfish farmers and resource managers alike to evaluate the consequences of increasing shellfish culturing activity within estuarine systems. It is becoming increasingly clear that the specific dynamics of nutrient fluxes, and the cumulative biomass of organisms dependent on those fluxes, can dictate both direction and magnitude of ecosystem level impacts to the marine environment (Herli, 1998).

The existing literature examining the linkages between intensive shellfish culture and the physical environment has focused largely upon either bottom culture of bivalves or suspended culture systems located in embayments which vary significantly in the degree of physical coupling between oceanic and estuarine circulation patterns. A general feature observed wherever bivalves have been cultured very intensively, either in circulation restricted embayments or within intensive grow out systems, has been a tendency for a depression of growth rates and other negative production factors associated with the over capacity of the growing systems at different spatial scales (Grant et al., 1993, due mainly to the removal of seston by the cultured mussels themselves (Navarro et al., 1991; Grant, 1999). Suspended mussel culture in the Benguela system (South Africa) has been recently cited as an example of a system in which extremely rapid mussel growth on an annual basis appears tightly coupled with oceanic upwelling which fuels, via nitrate input, high primary production in surface waters where mussels are grown (Monteiro et al., 1998; Grant et al., 1998). On the other hand, extreme nutrient limitations due to basin and bathymetric configuration, stratification events and/or hydrographic restriction on the annual flux of nutrients can result in the greatly reduced rates of growth in shellfish - as has been seen for many years in the intensive intertidal oyster culture operations on the Brittany coast of France (Herli, 1987; Barille et al., 1997).

Examining suspended shellfish culture at a more intimate spatial scale, suspension feeding bivalves have shown a high capacity to impact local seston concentration. Mussels grown at high densities can result in the local depletion of seston and a reduced scope for growth in the vicinity of growing structures (Navarro et al., 1991). Local depletion of seston was observed to be significant in the vicinity of cultured mussels within the raft systems in the Spanish rias (Perez Camacho et al., 1991). Similarly, cultured mussels in the Benguela system were observed to deplete seston in the vicinity of grow-out rafts; in this case thermal stratification caused reduced availability of nutrients above the thermocline in the vicinity of the mussels. In addition, the level of seston depletion was dependent on the mussel line density (number and spacing of mussel growing lines), and the age and size of mussels (Heasman et al., 1998). For bottom-cultured mussels, a similar relationship has been found with growth in the middle of dense assemblages of mussels being less than that on the edge of patches (Newell, 1990). Mussel growth was observed to vary greatly between sites, with seston flux being the dominant factor dictating growth rate for a variety of species and sites (Van Erkman Schurink and Griffiths 1993; Newell et al., 1998). Thus, it is clear that measurements of seston flux and their effect on mussel growth are critical in the evaluation of the ecological linkages existing between dense assemblages of suspension feeding bivalves and the rate of water column turnover in the surrounding marine environment. This relationship has been formalized as a Seston Depletion Index (SDI), where % seston reduction is related to filtration capacity as these factors both relate to the exchange of water in contact with filter-feeding bivalves (see Wildish and Kristmanson, 1997).

Seston limitation within the spatial scales appropriate to suspended culture systems can occur as either qualitative or quantitative reduction in the food supply itself. Changes in the ratio of phytoplankton to detritus, the organic to inorganic seston load or the total particulate material available can result in nutritional limitations to suspension-feeding bivalves (Bayne and Newell, 1983). Studies to assess the physiological energetics of suspension feeding bivalves have been based on measurements of the seston flux over and through mussels growing either in suspended culture (Navarro et al., 1991) or in bottom culture (Newell and Shumway, 1993). These measurement techniques, using standard methodologies, have been developed to investigate in particular the relationship between the physiological growth parameters, including scope for growth, and the ambient food availability available for marine bivalves (Bayne and Widdows, 1978; Widdows, 1984; Iglesias et al., 1998; Grant and Bacher, 1999).

Numerous studies making measurements of carrying capacity have used time-averaged estimates of flow through culture systems based on tidal exchange or short-term measurements of flow regimes adjacent to the study region. These studies suffer from several drawbacks, principally relating to the unique hydrodynamics normally associated with shellfish growing in suspended culture such that concurrent measurements of seston flux and feeding are required (see Wildish and Kristmanson, 1997). In addition, the hydrodynamic variability of water flow in and around feeding shellfish, and the boundary layer effects present at the interface of the animals and growing structures, especially at low flow velocities, must be considered. Unique hydrodynamic conditions different from those in adjacent areas have been associated with both mussel beds and rafts in Maine (Newell, 1990). In this case,
flow of water through a mussel raft was affected in three ways: 1) most flow diverged around the raft; 2) flow within the raft tended to be aligned with the ambient current direction; and 3) flow within the raft was reduced relative to flow outside the raft. Specific forcing factors regulating flow within the raft structure around the growing lines included the spacing of grow out lines, the overall velocity of currents, and the age and size of mussels (Boyd and Heasman, 1998). Food depletion or limitation at the boundary layer immediately adjacent to feeding bivalve shellfish can also reduce the rate of growth (Frechet and Bourget, 1985a), especially if the velocity of ambient currents is reduced to allow depletion of the food supply in the immediate vicinity of individual bivalves (Wildish and Kristmanson, 1984, 1985).

Many studies of bivalve feeding have focused on the determination of clearance rates, using either natural assemblages of phytoplankton or cultured algae, to make inferences about feeding activity under ambient conditions (Bayne and Newell, 1983; Widdows, 1984). Drawbacks to these methodologies include the difficulties associated with emulating natural conditions of shellfish feeding at depth and the need to measure particle depletion under conditions of varying seston quality and quantity (Doering and Oviatt 1986; Cranford and Gordon, 1992; Figley et al., 1992). Better understanding of bivalve feeding physiology and improved methodology make it possible to utilize the inorganic fraction of the seston as a tracer to calculate the amount of seston filtered from the water column by individual animals by making quantitative collections of pseudofeces and feces over a time interval (Davis, 1994; Iglesias et al., 1998). Routine sampling of seston in the immediate vicinity of shellfish and quantitative collections of feces and pseudofeces over a period of hours or days allow an accurate appraisal of both feeding rate and absorption efficiency in mussels and other suspension feeding bivalves (Davis, 1994) and eliminates the need for the calculation of clearance or filtration rates for individual bivalves.

Puget Sound is characterized by high flow rates; high natural nutrient levels; relatively constant and low water temperatures; and large freshwater influxes. There is little experimental information regarding the relationship between intensive raft-based aquaculture and the potential impacts of nutrient depletion on the ecology of the near-shore pelagic and benthic marine environments at any spatial or temporal scale nor have estimates of the carrying capacity of Puget Sound embayments used for intensive shellfish culture been made. Because seston contacts suspension feeders such as mussels through diffusion and advection relating to the specific flow characteristics inherent to a specific culture system (Grant et al., 1998), an understanding of specific water exchange and circulation patterns within, and in the immediate vicinity of, the cultured bivalve raft are essential in order to begin building a carrying capacity model for bays with ongoing or planned intensive bivalve culture (Insez and Lutz, 1980; Dame and Prins, 1998). Efforts to model shellfish populations under a variety of flow regimes have greatly augmented the ability to predict carrying capacity criteria for bivalve populations (Carver and Mallet, 1990; Newell et al., 1998). Using various methods, the effect of shellfish farming on the carrying capacity of growing waters have been studied with respect to seston depletion within a long line system (Insez and Lutz, 1980), resource allocation (Rodhouse et al., 1984, 1987), energy flow (Rosenberg and Loo, 1983), and physiological energetics (Navarro et al., 1991). An understanding of the local effects of suspended shellfish on water column flow dynamics, the associated depletion of seston by cultured shellfish populations, localized resuspension of ingested materials, biodeposition of feces and pseudofeces, and incorporation of material as tissues (with removal from the system through harvest) are important to assess at the scale of the commercial system. Carver and Mallet (1990) for example, examined the available food supply and food demand to yield a total mussel biomass of 200-600 tons as the maximum carrying capacity of a small (0.5 km²) semi-enclosed bay in Nova Scotia. It has been recently pointed out that even slight depletion of seston in the vicinity of feeding mussels can impact growth rate (Grant, 1999), which amplifies this earlier work in which "carring capacity", as the term relates to suspension-feeding bivalve culture, was defined as the stocking density of mussels which results in a 50% depletion in seston (Carver and Mallet, 1990).

These types of measurements will be required before larger scale impacts (e.g. regional effects of suspended shellfish farms) can be assessed, and the means to predict the sustainability of intensive culture and its effects on the local and regional marine environment are critical to assess for the Puget Sound ecosystem (see Dame and Prins, 1998). These measurements are considered essential for the continued development of the mussel aquaculture industry and the determination of the overall carrying capacity of Puget Sound embayments for mussel and other bivalve farming efforts. Further development and testing of models based on current flux and seston depletion by cultured bivalves need to be refined for types of commercial shellfish culture systems other than bottom culture (see Newell, 1999), and in time as a means for developing a sustainable aquaculture industry based on real-time measurements of food supply and depletion.
Contributions the study will make to the discipline

Reporting for this project will address changes in plankton density and suspended particle composition associated with shellfish filter feeding and potential carrying capacity effects. Specific products will include comprehensive baseline data, derived from intensive long-term and replicated field observations on heavily instrumented aquaculture raft and longline systems. These data will markedly improve our understanding of the inter-relationships of physical, chemical and biological processes in large-scale suspended culture systems, and will enhance our ability to predict the sustainability and carrying capacity of these systems. Project findings will be published in peer-reviewed journals and presented at pertinent meetings and conferences.

Contributions the study will make toward addressing the problems identified in the National Marine Aquaculture Initiative

Suspended mussel aquaculture is widely practiced in many areas of the world, particularly Japan, China, France and Spain. Presently, suspended systems of all types account for an estimated 25% of the total quantity of shellfish produced in the U.S. west coast. Many shellfish growers and shellfish biologists believe there is an excellent opportunity for increased production in inshore and offshore regions of the U.S. coast. However, this potential will not be realized unless critical issues regarding carrying capacity, and water quality and ecosystem effects of suspended shellfish are addressed. Research that evaluates these issues is essential for the formulation and application of regulatory guidelines and policies that will accurately reflect the impacts of this farming method. This has great implications where intensive shellfish culture is carried out for the production of a food crop or where it is proposed for water quality enhancement and waste treatment. Information provided by this project will be of significant benefit not only to the shellfish industry, but also to the agencies and organizations involved with the management of salmon and other estuarine resources. While this project will be confined to the U.S. West Coast, the study results will be pertinent elsewhere in the U.S.

Research Plan

Based on existing research and our understanding of suspended bivalve culture systems, it is clear that potential water column effects can range from little or no change in existing conditions to significant alterations in current flows and chemistry, and seston concentration and composition. Characterization of these changes is essential for the siting and evaluation of new culture facilities and in improving yield and production of existing facilities. The overall goal of this project is to quantify water column characteristics and effects of suspended shellfish culture associated with feeding and biodeposition by examining linkages between oceanographic and biological processes including the relationship between seston resources and current flux in commercial-scale systems. The following objectives build on existing research completed in the last ten years. These objectives are shown in context of the overall research strategy in Figure 4. While several shellfish species are cultured with suspended systems on the U.S. west coast, this research project concentrates on raft cultured Mediterranean, Mytilus edulis galloprovincialis, and native, M. edulis trossulus, mussels. The study objectives are as follows:

- **Objective 1:** To characterize under varying treatments of size and seasonality, bivalve shell growth and meat yield against measured physical, chemical and biological parameters including the flux of suspended particulate materials in the water column at a commercial mussel raft-based aquaculture system.
- **Objective 2:** To characterize a suite of physical, chemical and biological variables within an suspended culture system with concurrent physiological measurements of feeding and biodeposit production.
- **Objective 3:** To examine in comparative experiments the application of measured water quality and biological conditions on growth and yields in a production scale suspended bivalve system at a differing growout location.
- **Objective 4:** To collaborate with an on-going large-scale tidal flow and nutrient modeling study to evaluate the potential carrying capacity of intensively cultivated suspended bivalves in an entire farming area.

The proposed research will be directed by the Pacific Shellfish Institute (PSI), a non-profit shellfish research organization based in Olympia, Washington. Our project team has extensive experience in the project area and elsewhere on the U.S. west coast and is uniquely suited to conduct this research. The study sites have established commercial-scale mussel production and are readily accessible from the PSI office. Our grower partners are providing a significant level of logistic and laboratory support and access to their culture systems. Co-investigators also have extensive laboratory and field capabilities which are bring brought into the project as well.
Plan of Work

Objective 1: To characterize bivalve shell growth and meat yield against measured physical, chemical and biological parameters at a commercial mussel raft-based aquaculture system.

Research under this objective will be carried out in the 2001-03 period at a Puget Sound, Washington shellfish culture site (Totten Inlet in southern Puget Sound; see Figure 1) where high-density mussel (Mytilus edulis galloprovincialis) cultivation is practiced utilizing 10 m x 15 m rafts (Figure 2). These rafts are anchored in two farm locations at 10 to 15 m of water depth. They are fixed in the water column and do not rotate with changes in current direction. The initial phase of research will be carried out beginning in fall 2001 using replicate rafts (N=3) holding juvenile (6 month old, approximately one million mussels with a biomass of about 5,000 kg) mussels on grow-out lines (740 drops @ 3 meters per drop). Beginning spring 2002 a second replicate (N=3) group of rafts will be brought on line. These will be structures holding newly seeded mussel holding approximately one million mussels with biomass of about 50 kg. Research will continue through the project year, followed up, pending available funding, with a second phase in fall 2002 to include sampling on a new juvenile group at a second site in north Puget Sound (see Table 1 and Objective 3).

Published studies: Impacts of suspended shellfish culture; food utilization at raft level; feeding rates of shellfish; and plankton/sonet composition.

Objective 1: Characterize bivalve shell growth and meat yield against measured physical, chemical and biological parameters at a commercial mussel raft-based aquaculture system.

Objective 2: Characterize a suite of variables with concurrent physiological measurements of feeding and biodeposit production.

Available regional data: Water currents, weather patterns, nutrients, chlorophyll, DO, temp, plankton.

Objective 3: Examine the application of measured water quality and biological conditions on growth and yields at a differing grow-out location.

Food uptake, and seston/plankton research: Application of procedures and available instruments in central and north Puget Sound, Hood Canal, Oregon, Alaska, and to other species.

Objective 4: Collaborate with a large-scale tidal flow and nutrient modeling study to evaluate carrying capacity of suspended culture systems.

Carrying capacity studies: Applications for projecting siting and expansion in the region and elsewhere.

Figure 4. Formulation of a farm-scale carrying capacity estimate and quantification of effects. Numbered boxes with borders refer to specific objectives of this proposal.

National Marine Aquaculture Initiative -- Ecological Characterization Page 7
Analytical methods

Environmental sampling will be conducted as described below along with studies of the growth and yield of the cultured mussels. The following analytical methods will be used:

Water currents: A acoustic (Soncte or similar brand) doppler velocimeter (ADV) will be used to measure 3D water velocity. A total of six matching ADVs will be deployed to measure water velocity upstream, downstream and within the raft, and at an adjacent reference station. The ADVs employed for the project will use acoustic doppler technology to measure 3D flow in a small sampling volume located a fixed distance from the probes. The velocity range and data acquisition rate are programmable. The instruments will be programmed to acquire data at a sampling interval of 5 to 15 minutes. Northwest Research Associates will be providing instrument deployment and data analysis support, and 5 of the 6 ADVs during the first year.

Tides and weather: Meteorological and tidal data will be obtained to identify important trends which could affect water current flow and direction, and water quality at the study site. Meteorological data will be collected from available weather instrumentation. These data will include wind speed and direction, barometric pressure and air temperature. Tide data will be computed with tide prediction software.

Water quality conditions: Most temperature, dissolved oxygen, salinity, turbidity and chlorophyll data will be obtained in situ with automated data logging sondes (YSI 6600 or similar). Additional information will be recorded by PSI developed dataloggers. These are built by PSI “in-house” and were designed for use in an ongoing PSI study, funded under the NOAA-ODRP program. They are robust, multi-parameter data loggers capable of intertidal and high subtidal deployments (<15m depths). The parameters currently being measured include: dissolved oxygen; salinity; temperature; and depth, although up to five other sensing circuits can be added as future needs warrant. Both instruments will log all of the parameters at 5 to 15 minute intervals and store readings for later downloading.

The YSI sondes will also be used in real-time mode, combined with a flow-cell and automated or manual water samplers (see below) to obtain water quality data at multiple points from within each raft unit. Water quality data obtained with the in-situ data loggers will be calibrated against split samples analyzed in the laboratory. Water samples for nutrient analysis will be obtained with automated or manual water samplers (see below) and analyzed by the water chemistry laboratory at the University of Washington Department of Oceanography. Orthophosphate, silicates, nitrate/nitrite nitrogen, ammonia and total dissolved phosphorus will be analyzed using standard spectrophotometric methods.

Phytoplankton, zooplankton and seston: Phytoplankton, zooplankton and seston will be collected by pumping from depth using a 12 volt peristaltic pump and an automated raft-mounted water sampler (Sigma Model 900 or similar) into 1 to 10 1 bottles containing a preservative. Multiple samplers and/or multiple sample manifolds will be used to obtain samples from the perimeter, center and bottom of the raft system. Each sampler will be programmed to collect one sample/12 hour to one sample per day, depending on the experimental protocol. Phytoplankton and zooplankton will also collected by routine vertical hauls using a 0.25 m diameter 20 μm mesh plankton net. The live, net-caught phyto- and zooplankton will be screened on-site for determination of major taxa and rough composition and preserved for laboratory identification. Biological samples will be returned to the laboratory for seston and plankton analyses. The analysis for total seston will follow standard methods (Strickland and Parsons, 1972). Sea water samples (1-10 liter) collected by pumping from depth will be vacuum filtered through pre-ashed and pre-weighted glass fiber filters (GF/C), rinsed with isotonic ammonium formate using gentle vacuum and dried to a constant mass (24 h @ 70 °C), weighed a second time and combusted (4 h @ 450 °C) prior to a final weighing. Particulate organic material (POM) or total volatile solids (TVS) and particulate inorganic material (PIM) will be determined by difference. Dr. Kenneth Brooks from Aquatic Environmental Sciences will be assisting the project team in these analyses.

Phytoplankton and zooplankton samples from automated and net sampling will be examined using phase-contrast light microscopy to generate a species list and determine species relative abundance (dominant, many, few). Quantitative water bottle samples will also be examined using inverted microscope techniques where a portion of a sample is settled in special chambers and the cells are counted. Dr. Rita Horner at the University of Washington School of Oceanography will be providing support to the project team for phytoplankton taxonomic classification and confirmation.
Mussel growth and biomass: Measurement of changes in growth and biomass of cultured mussels will be determined as a change in shell length and ash-free dry tissue weight over each seasonal sampling time frame, and for the entire grow-out cycle. Mussels (n = 50 per raft) will be gathered every 2 to 4 weeks with no size selectivity from drop lines upstream, center and downstream portions each raft and returned to the laboratory. Shell measurements will be taken (shell length, height and width) on individual mussels within each sample. Valves will be cleaned of epibions which will be saved for later taxonomic analysis of fouling organisms over the period of growth out (see below). Mussel tissues will be oven dried, weighed and combusted (400 °C for 4 hours) and reweighed to obtain an ash-free dry mass for sampled tissues.

Fouling: Because fouling of mussels occurs as a matter of course over the 18 month growout period for mussels at this site, replicate blocks of mussel drops within the experimental systems will be cleaned of epibions and compared to blocks of mussel drops left uncleaned to enable an estimate of the effect of invertebrate and other fouling on flow and seston availability to mussels. This fouling will consist largely of algae, tunicates, sea anemones, barnacles, bryozoans and caprilliid amphipods. All fouling organisms will be used to generate a species list, relative abundance and wet weights of each taxa group. Dr. Kenneth Brooks from Aquatic Environmental Sciences will be assisting the project team in sampling and classification of fouling taxa.

Experimental Design and Sampling Schedule

Each group of replicated rafts (3 replicates for each treatment) will be sampled over a 2 week period during each of four sampling sessions (May, August, November and March) as shown in Table 1.

Table 1. Sampling schedule for mussel raft systems at Totten Inlet (Objective 1) and Penn Cove (Objective 3) systems.

<table>
<thead>
<tr>
<th>Project Year</th>
<th>Sampling Season</th>
<th>Year 1</th>
<th>Year 2</th>
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<tr>
<td></td>
<td>Fall</td>
<td>Winter</td>
<td>Spring</td>
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<tr>
<td>Totten</td>
<td>J</td>
<td>J</td>
<td>S</td>
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<tr>
<td>Penn Cove</td>
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S = seed mussels at start of study; J = juvenile mussels (6 mo old) at start of study
'Totten = Deepwater Cove, Taylor Shellfish Farms, Penn Cove = Whidbey Island, Penn Cove Mussels

Water parameter profiling instrumentation will be deployed and sampling will be carried out on the perimeter and mid sections of rafts at specified stations and at the approximate mid-line depth of the mussels on individual rafts for measurement of water quality variables and site specific flow characteristics. Simultaneous sampling for total seston, phytoplankton, and zooplankton will be conducted for long-term measurement of the flux of seston available to mussels in the raft system. Water mass profiling will also occur beneath the rafts and at a nearby reference station to characterize the native flow regime, water quality, plankton and seston content for comparison to the raft environment. A generalized diagram of the proposed sampling plan is shown in Figure 5.

Logistics, instrumentation and cost limitations will prevent simultaneous observations of all replicated rafts, therefore we propose to conduct combined automated water current and in-situ water quality sampling for a total of 2 weeks per seasonal sample period at one of the replicates, with intensive sampling for other parameters using manual and automated techniques at all three replicates during the same 2 week period. For example, during the spring 2002 sampling in Year 1, we would operate the automated instruments for 2 weeks on one of seed culture rafts, and then move the equipment to the juvenile rafts for another 2 weeks of monitoring. Sampling for phytoplankton, seston and mussel growth/yield will be taken over a 4-week period covering the period of seasonal sampling. During the periods between the seasonal intensive sampling, we will continue to monitor water quality and biological parameters at a reduced frequency from one reference station located adjacent to the culture system.

During periods of high sample rates on all rafts, seston, plankton and water samples will be taken once a day at slack water. This will allow a comparison vs tide states across the seasons without the added complexity of current flow. Sampling at this intensity will create large number of samples (14 days x 9 stations = 126 s x 3 rafts = 378 samples). These will be processed and analyzed during the time periods between the seasonal sampling. Total production of mussels on mussel drops will be assessed at two week intervals for each raft during the intensive study.
Data collected on flow vectors and seston content at multiple points within replicate raft systems will be integrated to yield the flux of available sesten, including resuspended materials, available to mussels at different positions within the raft system under specific tidal flow conditions as a function of age/size/weight of mussels over grow out cycle. In addition, information on fine-scale flow patterns as they relate to the topography of mussel drops will be mapped over time as a function of tidal flow rate and direction, and mussel biomass, which will help characterize both advective and convective sesten flux processes within and adjacent to individual mussel rafts. Data collected will be used to construct a time series analysis of sesten removal rates, biodeposition rates by mussels and associated fouling organisms and flow characteristics of water masses within and adjacent to mussel rafts during a typical grow out cycle for cultured mussels.

Objective 2: To characterize a suite of physical, chemical and biological variables within a suspended culture system with concurrent physiological measurements of feeding and biodepositon production.

The observations made in Objective 1 will generate a detailed picture of the behavior of the raft culture system over the entire grow-out period. In conjunction with Objective 1, we will measure mussel feeding and biodeposition rates in situ during full tidal cycles over two week periods four times annually. These studies will immediately follow the intensive raft-scale sampling described above. They are an essential component of this research for identification of within raft biodeposition and feeding rates.

We will use the quantitative biodeposition method (Cranford and Hargrave (1994), Davis (1994) and further tested by Iglesias et al (1998)) to determine both seston removal rates by mussels and biodeposition rates of fecal and pseudofecal material production at ambient flow rates and seston levels. Ingestion and absorption rates are determined by measuring biodeposition rates in mussels and the proportion of organic to inorganic material in feces, pseudofeces (if produced) and the seston over a suitable time interval. Clusters of mussels cut from randomly selected drop lines will be placed in “biotraps” similar in design to that described by Cranford and Hargrave (1994) (see Figure 6), lowered to specific depth and position in the raft system and allowed to equilibrate to ambient conditions. These “biotraps” or biodeposit collection boxes will be oriented parallel to the existing flow regime within the instrumented juvenile raft (1 of the 3 replicates), and will have a diameter large enough to reduce boundary layer affects associated with structure (see Wildish and Kristmanson, 1997). Replicate groups of mussels will be placed in identical biotraps (n = 10) at other points within the raft concurrently. Multiparameter sondes and
multi-directional flow meters described under Objective 1 above will be placed adjacent to individual bioraps to measure flow rate and direction through the raft at the point of measurement, and a sampling port will allow the pumping of sea water samples to the surface for measurement of total seston. Biodeposits from feeding mussels will be collected quantitatively. Sampling will consist of pumping accumulated biodeposits by peristaltic pump to the surface from the trap over a specific sampling interval for collection of the material onto GF/C filters for later analysis (see analytical details above) Because ash is not absorbed during digestion in bivalves, the rate of ash ingestion can be simply computed as a function of the proportion of ash in feces (Eash) to ash in the food (Fash) derived from the measure of PIM in the seston. Therefore, the rate of ingestion (IT) may be computed as follows:

\[
IT = \frac{Eash}{Fash} \text{ (from Cranford and Hargrave (1994))}
\]

Similarly, we will estimate absorption efficiency (AE) from the same parameters according to the following:

\[
AE = 1 - \left(\frac{Fash\text{Absorbed}}{Eash\text{Absorbed}}\right) \times 100 \text{ (from Cranford and Hargrave (1994))},
\]

where Fabsorbed is the organic fraction of the food and Eabsorbed is the organic fraction of the feces, respectively.

![Diagram of biotrape chamber](image)

**Figure 6.** Generalized diagram of the biotrap chamber to collect biodeposits from mussels placed within the raft system.
We will also make comparisons of feeding and absorption efficiency between mussels scrubbed clean of epibionts (e.g. barnacles, bryozoans, sponges and other suspension feeders) and shellfish fouled with these organisms in order to simulate differences in observed flux measured in the field. Care will be taken to simulate as closely as possible the boundary layer constraints associated with filter-feeding organisms by utilizing clusters of mussels in the biotrap to replicate natural clumping of mussels on grow out lines. Individual mussels in each cluster will be measured (shell length) and tissues removed, dried and ashed (400 °C for 4 hours) to determine tissue ash-free dry mass. Feeding and biodeposition rates for mussels will be expressed as mg seston consumed or biodeposits produced per hour per gram dry tissue weight. Feeding and biodeposition rates will be corrected to a standard body mass using standard regression equations for the allometric correction of differences in ingestion/biodeposition rates in mussels of varying size (see Widdows, 1984).

**Objective 3:** To examine in comparative experiments the regional application of measured water quality and biological conditions on growth and yields in production scale suspended bivalve systems at a differing growout location.

The intensive observations made for Objective 1 and experiments conducted for Objective 2 should have value beyond the immediate farm area tested. Objective 3 is a comparative evaluation of a similar large-scale suspended mussel raft system operated by Penn Cove Mussels, Inc. located in Penn Cove, on Whidbey Island, in northcentral Puget Sound. This farm is the largest west coast producer of mussels, primarily of the native, *M. edulis trossulus*, species. The farm site is located in relatively exposed waters and has differing water quality, food availability, and grow-out conditions from the Totten site. Research at this site will further test and develop tools for application in the less-intensive mussel and scallop long-line, and oyster pearl and lantern net systems found elsewhere in the region. This work will be conducted in the second project year (FY 2003). The study methods and approach will parallel those described for Objective 1, with the sampling schedule as shown in Table 1.

**Objective 4:** To collaborate with an on-going large-scale tidal flow and nutrient modeling study to evaluate the potential carrying capacity of intensively cultivated suspended bivalves in an entire farming area.

South Puget Sound (located south of The Narrows near Tacoma, Figure 2a) is divided into numerous blind-end inlets which, while having poor circulation, also possess ample shorelines and rural associated watersheds making them attractive places to live. In the past decade, many of the stream corridors and shorelines in the area have experienced considerable residential development. In 1990, Thurston County ranked as the third fastest growing county in Washington with a population increase of nearly 30% since 1980 (personal communication, Thurston County Planning Council, 1996). Development is expected to continue at a high rate in the counties containing the major drainage area of South Sound (i.e., Thurston, Mason, and Pierce Counties). As a result of increases in human activities, nutrient loading to the South Sound is likely greater today than it has been in the past, and will likely be even greater in the future. The Washington State Department of Ecology (DOE)'s Southwest Regional Office is concerned about point and non-point source nutrient loading to the southern portion of Puget Sound. Their major concern being that the current and future growth around this region may cause adverse effects on water quality due to increased eutrophication.

In an effort to better understand the dynamics of nutrient fluxes and loading within the southern part of Puget Sound the Department of Ecology is currently working on the Southern Puget Sound Model Nutrient Study (SPASM). This model addresses all of Southern Puget Sound, including Totten Inlet, but has enough spatial resolution to be of benefit to the present analysis and can provide the best currently available estimates of flushing rates in Totten Inlet.

While the designers of the SPASM model were originally focusing on the study of nutrient loading within southern Puget Sound, they feel that the model is flexible enough to tolerate being adapted to evaluating carrying capacity parameters. The current meter data and the vertical profiling information obtained in this project will be used by the verify the model and revise local current flushing and flow rate estimates. Our definition of carrying capacity is defined as a 50% seston reduction by feeding bivalves in intensive culture. This carrying capacity analysis will utilize water mass flushing data from the project site and SPASM, published information on plankton replacement times, and the particulate data obtained during this study to assess the influence of the experimental raft system on carrying capacity in the local area. Using conservative estimates for filter-feeding biomass in the study area and the bivalve biomass in the culture system we will then evaluate the probable impact of a larger-scale suspended farm operation on the potential carrying capacity of the given system (see Grant et al., 1998). Bivalve filter feeder "pumping" formulae will also be used to compare measurements made in the field to literature values for feeding in mussels and major producing systems reported by Dame and Brins (1998). Information detailing the effects of large-scale shellfish aquaculture and water quality enhancement activities on flow characteristics and net nutrient
removal (via plankton uptake and subsequent harvest) within embayments will also be used in refining the model. Research for this objective will be conducted during the second project year (FY 2003).

Roles of Project Personnel

- Daniel Cheney, Pacific Shellfish Institute -- responsible for directing the overall work effort and serving as the primary liaison with Sea Grant’s grant officer and the general public; jointly responsible for field and laboratory experiments in Puget Sound; biological characterization of culture system, and reporting; and ensuring completion of the tasks to PSI’s and Sea Grant’s satisfaction.
- Jonathan Davis, Taylor Resources, Inc. -- seston flux modeling and reporting; jointly responsible for field and laboratory experiments in Puget Sound; shellfish farmer liaison.
- Kenneth Brooks, Aquatic Environmental Science Lab -- epifaunal sampling, analysis and reporting; biodeposit analysis and evaluation.
- Frank Smith, Northwest Research Associates, Inc. -- water current monitoring and data summation, current meter deployment and maintenance.
- Curtis Ebbesmeyer, Evans-Hamilton -- physical oceanography, south Puget Sound modeling; collaboration with Washington Department of Oceanography staff oceanographers.
- Rita Horner, University of Washington -- phytoplankton taxonomic analysis; staff training and post-processing assistance.

Output and Economic Benefits

Suspended mussel aquaculture is widely practiced in many areas of the world, particularly Japan, China, France and Spain. Only recently has this form of aquaculture become a significant factor in shellfish production in the U.S. West Coast where production levels near $2,000,000 are modest, but growing. Many shellfish growers and shellfish biologists believe there is an excellent opportunity for increased production, but this potential will not be realized unless critical issues regarding carrying capacity and water quality impacts of mussel farming are addressed. A better understanding of these issues will have a great predictive value for shellfish farmers and regulatory agencies alike as future farm development issues arise.

This project will also help to further refine a regional model that will contribute to a better understanding of the dynamics occurring within a complex body of water.

Reporting for this project will address changes in plankton density and suspended particle composition associated with shellfish filter feeding and potential carrying capacity effects. While the results will apply specifically to the suspended systems under study, the general conclusions should be appropriate for bottom and off-the-bottom culture and proposed bivalve-based water quality mitigation projects as well. Specific products will include baseline data, derived from real-time field observations, that can assist shellfish farmers, local and county Coastal Zone Management personnel, and Departments of Natural Resources, Ecology, Fish and Wildlife and other public entities in their efforts to assess the effects of mussel raft culture in the nearshore coastal environment. Project findings will be published in peer-reviewed journals and presented at pertinent meetings and conferences.

Coordination with other Program Elements

This project forms a complimentary effort to the Washington State Department of Ecology’s effort to further refine their SPASM nutrient model for southern Puget Sound. Current meter data and the vertical profiling information obtained during this project will be used by the DOE to verify their model and revise local current flushing and flow rate estimates within a single representative embayment. Information detailing the effects of large scale aquaculture activities on flow characteristics as well as the net nutrient removal (via plankton uptake and subsequent harvest) within embayments will also be of use in refining the SPASM model. Our carrying capacity analysis will utilize water mass flushing data from the project site and from the SPASM model, published information on plankton replacement times, and the particulate flux data obtained during this study to assess the influence of the experimental raft system on carrying capacity in the local area.

PSI is also engaged in Sea Grant funded Oyster Disease Research Program and USDA SBIR work which has related elements. Both studies utilize field sites in south Puget Sound with regular sampling of a variety of water quality and biological parameters (plankton, DO and temperature) in the intertidal areas of the region.
AQUACULTURE INITIATIVE REFERENCES


